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ERRATA AND AUTHORS' EMENDATIONS

Page 58, line 33, first column, "Pesudomonas" should read "Pseudomonas."
Page 58, footnote, line 2, "Yonemachu" should read "Yonemasu."
Page 70, Table I, line 13, for "Algoma, Miss." read "Algoma, Minn."
Page 91, second paragraph of "Summary" first line, "although disease" should read "although the disease."
Page 151, Table VIII, last column, first entry "40–43" should read "40–42"; same column, last entry "30–22" should read "30–32."
Page 155, Table XI, in entry "II," last column, "May 3, all disappeared" should read "May 10, all disappeared."
Page 168, second column, end of first paragraph, "the growth s scanty and numerous, myceliumlike" should read "the growth is scanty and numerous, mycelial-like."
Page 186, first column, line 13, "measurement" should read "measurements."
Page 193, first column, line 32, "(100)" should read "(10)."
Page 210, first column, first paragraph, first sentence should carry a reference to: Carleton, M. A. A Serious New Wheat Rust in This Country. Science 42: 58–59. 1915.
Page 212, on map, "Pulman" should read "Pullman."
Page 225, first column, paragraph 1, line 7, "Observations made by writers in 1915" should read "Observations made by the writers in 1915."
Page 264, first column, line 14, "In 1899" should read "In 1889."
Page 349, first column, second paragraph, last line "Many of the seed which grew were over a hundred years old" should read "Some of the seeds," etc.
Page 384, line 7, "Y represents the weight of the liver in grams," should read, "Y represents the weight of the lungs and trachea in grams."
Page 392, after line 26, add: "In hybrid pigeons, Dr. Riddle found the left testis larger more frequently than in the pure species, but even in these, the left testis was larger in much less than 50 per cent of the cases."
Page 400, subhead, column 1, "Isolation of the Casual Organism" should read "Isolation of the Causal Organism."
Page 510, column 1, line 16, "organisms" should read "organism."
Page 513, column 2, lines 16, 17, and 18 should read, "and three forms of Botrytis associated with onion neckrot, *Botrytis allii*, *Botrytis* species 110, and *Botrytis* sp. 108 a."
Page 542, Table 11, column 4, line 5 "(*a*)" should read "*a* 0."
Page 549, first column, tenth line from bottom, "young hyphae can only dissolve" should read "young hyphae only can."
Page 575, second column, end of second paragraph, add following sentence: "This variation seldom being more than one degree."
Page 577, after Figure 3, insert "(Drawn by Adam G. Böving.)"
Page 578, title of Figure 6 should read: "*Epitrix parvula*."
Page 584, under *Summary:* first paragraph, should read as follows: "The tobacco crop * * * is attacked by the overwintered brood and by two, sometimes three, later generations. * * *"

JOURNAL OF AGRICULTURAL RESEARCH

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SEGREGATION AND CORRELATED INHERITANCE IN CROSSES BETWEEN KOTA AND HARD FEDERATION WHEATS FOR RUST AND DROUGHT RESISTANCE[2]

By J. ALLEN CLARK [3]

Agronomist in Charge, Western Wheat Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture

RESISTANCE TO RUST AND DROUGHT DESIRED

In the northern spring-wheat area of the United States drought and rust are the principal limiting factors in successful wheat production, and are the causes of enormous losses. Since certain varieties of wheat have been found to possess resistance to these destructive agencies, the problems for further improvement are important to the wheat breeder.

The writer here presents some early studies made in a cross between Kota, a rust-resistant variety of hard red spring wheat, and Hard Federation, a drought-resistant variety of white wheat. The effect of this resistance on yield is of great economic importance. High yield, however, is not the only consideration; the quality of the product is of equal importance to that of yield. A careful study of the milling and baking qualities of the parents and hybrids, therefore, is included in the present work.

MATERIAL AND METHODS

A new variety of hard red spring wheat in order to be successful must yield more than the principal competing commercial variety and be equal or superior to it in milling and baking qualities. The most extensively grown variety of hard red spring wheat at the present time is Marquis. The value of the parents and hybrids in this study, therefore, will be determined in comparison with the Marquis variety.

SELECTING THE PARENTS

Kota and Hard Federation were selected as parents for the cross here discussed because they appeared to supply the best available material for combining in one study the problems of breeding for both rust and drought resistance. The reasons which influenced the selection of the varieties used are their high yielding ability under conditions of rust and drought, and the milling and baking qualities of both varieties. They have several contrasting morphological characters and milling and baking qualities which contribute to their value. The inheritance of these is important.

Both varieties are comparatively new to the farmers of the northern spring-wheat area of the United States. They are new even to the experimental agronomist. Neither has been included in varietal experiments for longer than six years. The writer believes, however, that they have been sufficiently tested to insure their successful commercial production over wide areas as well as their successful use in the mill and bakery. The commercial production of both varieties in the United States began in 1919 and nearly 50,000 acres of each were grown in 1923.

KOTA

Kota is a variety of hard red spring wheat which is resistant to black stem

[1] Beginning with Volume 29, No. 1, the Journal of Agricultural Research will appear semimonthly instead of weekly, the issue dates being the 1st and 15th of each month.

[2] Received for publication Mar. 29, 1924—issued January, 1925.

[3] The writer wishes to express his appreciation to Dr. Sewell Wright, of the Bureau of Animal Industry, and to Dr. H. K. Hayes and Dr. Fred Griffee, both of the Minnesota Agricultural Experiment Station, for advice and assistance given during the collecting and analyzing of the data. To V. H. Florell, at Davis, Calif., Olaf S. Aamodt, at St. Paul, Minn., and J. C. Brinsmade, jr., at Mandan, N. Dak., thanks are here gladly given for assistance in growing the material studied and in taking notes thereon. The writer also desires to acknowledge the services of John R. Hooker for statistical assistance given.

rust. The resistance in this variety was determined independently in 1918 by Waldron and Clark (*36*).[4] The original seed of the variety was obtained in Russia by Prof. H. L. Bolley, of the North Dakota station, in 1903, while making a study of the flax industry of Europe for the United States Department of Agriculture. Previous to 1918 resistance to stem rust of wheat was a quality not known to have been reported among varieties of hard red spring wheat grown in the United States.

Kota has awned, fusiform spikes; glabrous, white, glumes; and midlong, hard red kernels. It is a midtall, midseason spring wheat which has weak to midstrong stems.

For a more complete description of both Kota and Hard Federation, see Clark, Martin and Ball (*6*).

ADAPTATION.—The acre yields of Kota have averaged considerably higher in North Dakota and South Dakota during the past three to five years than those of Marquis, the standard variety of hard red spring wheat. Kota also has produced good yields in the northeastern portions of Montana and Wyoming. It has proved somewhat resistant to drought, as well as distinctly resistant to stem rust, in the northern Great Plains area.

In the more humid sections of the spring-wheat region, including Minnesota, Wisconsin, and Iowa, Kota is not well adapted. Experiments in these States show that Kota lodges readily, and principally for this reason it yields poorly and is not suited to humid conditions. From central Montana westward, Kota also has not proved to be particularly well adapted. In this intermountain and Pacific coast area varieties of common white wheat, especially Hard Federation, have considerably outyielded both Kota and Marquis.

Kota has been included in experiments at the North Dakota Agricultural Experiment Station near Fargo, N. Dak., for a longer period than elsewhere. Experiments at Mandan and Dickinson also show it to be well adapted to North Dakota. At Moccasin, Mont., however, which is outside of the area affected by stem rust, Kota is not better adapted than Marquis. The yields of Kota and Marquis obtained from replicated plat experiments at these four experiment stations serve to show the adaptation of Kota and are given in Table I, with the average difference in bushels, and per cent, the odds against the difference being due to chance as determined by Student's (*1*) method.

Additional yields and rust data reported by Clark and Waldron (*9*) give more complete information regarding the adaptation of Kota. Table I shows, however, that there is an important and significant difference between the yields of Kota and Marquis at Fargo and Mandan, but decreasing

TABLE I.—*Annual and average acre yields of Kota and Marquis wheat grown in experiments at four experiment stations during four or more of the six years from 1918 to 1923, inclusive*

Station and variety	Acre yield (bushels)								Odds	Per cent-age of Mar-quis
	1918	1919	1920	1921	1922	1923	Aver-age	Dif-fer-ence		
Fargo, N. Dak.:[a]										
Kota	28.4	17.4	22.8	17.8	30.8	29.2	24.4	+4.3	36.5:1	121.4
Marquis	21.5	15.7	12.2	19.4	26.1	25.5	20.1	------	---------	100.0
Mandan, N. Dak.:[b]										
Kota	------	------	9.1	4.4	18.6	14.4	11.6	+1.4	125.6:1	113.7
Marquis	------	------	7.4	3.8	16.7	12.9	10.2	------	---------	100.0
Dickinson, N. Dak.:[c]										
Kota	------	6.6	22.4	4.2	31.0	21.8	17.2	+2.2	9.1:1	114.7
Marquis	------	3.4	15.9	5.6	31.6	18.3	15.0	------	---------	100.0
Moccasin, Mont.:[c]										
Kota	------	------	21.0	28.3	26.5	23.8	24.9	−0.9	15.2:1	96.5
Marquis	------	------	23.1	28.3	27.3	24.6	25.8	------	---------	100.0

[a] Experiments conducted independently by the North Dakota Agricultural Experiment Station, the courteous permission to use the results being hereby acknowledged.

[b] Experiments conducted cooperatively by the Offices of Cereal Investigations and Dry-Land Agriculture Investigations of the Bureau of Plant Industry, U. S. Department of Agriculture.

[c] Experiments conducted by the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the State agricultural experiment stations.

[4] Reference is made by number (italic) to "Literature cited," p. 46–47.

in significance at Dickinson and westward as damage from rust decreases until at Moccasin, Mont., Kota is slightly outyielded by Marquis.

QUALITY.—Milling and baking experiments conducted in the United States Department of Agriculture from 1918 to 1923, inclusive, show Kota to compare very favorably with Marquis in these respects. In all, 86 samples of the Kota variety, representing five crop years, have been studied. These samples were obtained from experiment stations and commercial sources. The commercial samples do not make possible an accurate comparison with Marquis. At experiment stations, however, the two varieties were grown under exactly the same conditions and comparable samples were obtained for experimental milling.

TABLE II.—*Summary of milling and baking data from 86 samples of Kota wheat and from 60 of these Kota samples and 60 comparable samples of Marquis grown during the five crop years from 1918 to 1922, inclusive* [a]

Descriptive data	All Kota samples	Comparable samples		
		Kota	Marquis	Kota in percentage of Marquis
Number of samples	86	60	60	
Bushel weight, pounds	60.3	59.8	57.5	104.0
Crude-protein content of wheat [b], per cent	14.3	14.9	14.2	104.9
Yield of straight flour, do	74.2	73.4	71.4	102.8
Yield of shorts, do	14.1	14.7	14.2	103.5
Yield of bran, do	14.1	14.1	16.5	85.5
Milling gain, do	2.4	2.2	2.1	104.8
Water absorption of flour, do	64.9	65.5	60.1	109.0
Volume of loaf, cubic centimeters	2,242	2,246	2,272	98.9
Weight of loaf, grams	512	514	502	102.4
Texture of crumb, score	91.4	91.0	89.0	102.2
Color of crumb, do	90.1	89.9	90.8	99.0
Ash in flour, per cent	[c] 54	[d] 57	[d] 51	111.8

[a] Experiments conducted by the Milling Investigations Section, Grain Division, of the Bureau of Agricultural Economics.
[b] N×15.7, basis 13.5 per cent moisture.
[c] Average of 84 samples.
[d] Average of 58 samples.

Table II shows the average results from the 86 samples of Kota and from 60 comparable samples of Kota and Marquis and the difference between the comparable samples expressed in percentage of Marquis taken as 100 per cent.

The data show Kota to exceed Marquis in most factors. A statistical analysis of these results by Clark and Shollenberger (*7*) fully discusses the significance of the differences and variability. There are objections to the Kota variety by the trade because of its high ash content and the creamy or yellowish color of its flour. To meet these objections by the grain trade and maintain the good qualities of the variety are a part of the object of this investigation.

HARD FEDERATION

Hard Federation is a variety of white wheat which has proved very resistant to drought. It was developed about 1908 by J. T. Pridham, at the Cowra Experiment Station in New South Wales, Australia, by selection from Federation. It was introduced into the United States by the United States Department of Agriculture in 1915, and was first tested at experiment stations at Moro, Oreg., and Chico, Calif. The success of the experiments was reported by Clark, Stephens, and Florell (*8*). From these stations seed has since been distributed to farmers.

Hard Federation has awnless, oblong spikes; glabrous, brown glumes; and short, hard, white kernels. It is an early spring wheat which has very strong stems and small leaves that twist or curl. This latter peculiar habit undoubtedly is heritable. It apparently decreases transpiration, which is one cause of the resistance of the variety to drought.

ADAPTATION.—Hard Federation has proved to be the highest yielding variety of spring wheat grown under dryland conditions in Oregon. In California it is grown from fall sowing and is well adapted to some soils. It also has produced excellent yields in central

Montana. In these Pacific coast and intermountain States it often outyields Marquis from 5 to 10 bushels per acre, and it is rapidly becoming an important variety in many of the semiarid sections of the west. It is susceptible to stem rust and does not yield well where this disease is prevalent.

Hard Federation has been included in varietal experiments for a longer period at the Sherman County Substation, Moro, Oreg., than elsewhere in the United States. During the six-year period, from 1918 to 1923, inclusive, in which it has been grown there, the annual precipitation has averaged 11.36 inches. When grown on clean summer fallow under this limited rainfall, Hard Federation has proved a remarkably drought-resistant wheat. Eastward the advantage decreases, although at Moccasin in central Montana it has produced some very favorable yields in dry seasons and when weeds were not abundant. At Dickinson and Mandan, N. Dak., however, where stem rust, as well as drought, has limited the yields of wheat and where weed growth is abundant, Hard Federation has not shown to outstanding advantage. The short stems and curling leaves of Hard Federation render it unadapted for overcoming weed growth. The yields of Hard Federation and Marquis from varietal experiments at Moro, Moccasin, Dickinson, and Mandan, are shown in Table III, together with the differences in bushels, the odds against the occurrence of such a difference being due to chance alone, and the percentage of Hard Federation in terms of Marquis.

Table III shows that there is an important and significant difference in the yield of Hard Federation over Marquis in the drier western sections, but the difference decreases in amount and significance eastward.

QUALITY.—Milling and baking experiments have been conducted by the United States Department of Agriculture with Hard Federation wheat during the 5 years from 1918 to 1922, inclusive. In all, 49 samples have been studied. As many as 44 of these can be directly compared with comparable samples of Marquis. Table IV gives a summary of these results.

The data show Hard Federation to compare favorably with Marquis in most factors. For a white wheat it is shown in experiments reported by Shollenberger and Clark (10) to be one of the best varieties for breadmaking in its class. It has no important objectionable feature when compared with Marquis, the leading variety for milling and breadmaking. The ash in its flour is low in comparison with Marquis and the color of crumb is high. Partly for these reasons it was selected for crossing with Kota with the hope that selections of the hybrid could be developed which would relieve the objection of the trade to the Kota variety.

TABLE III.—*Annual and average acre yields of Hard Federation and Marquis wheats grown in experiments at four agricultural experiment stations during three or more of the six years from 1918 to 1923, inclusive*

Station and variety	Acre yield (bushels)								Odds	Percentage that Hard Federation is of Marquis
	1918	1919	1920	1921	1922	1923	Average	Difference		
Moro, Oreg.[a]:										
Hard Federation	21.3	28.7	25.9	29.0	21.0	40.8	27.8	+6.4	Even	129.8
Marquis	15.0	22.7	19.2	19.7	17.0	34.9	21.4			100.0
Moccasin, Mont.[a]:										
Hard Federation			30.8	32.1	34.2	[b]17.1	28.6	+2.8	13 to 1	110.8
Marquis			23.1	28.3	27.3	34.6	25.8			100.0
Dickinson, N. Dak.[a]:										
Hard Federation				3.0	29.7	9.7	14.1	−5.4	10 to 1	72.2
Marquis				5.6	31.6	18.3	19.5			100.0
Mandan, N. Dak.:										
Hard Federation				6.7	18.1	14.1	12.9	+0.2	2 to 1	101.1
Marquis				3.8	16.7	12.9	12.7			100.0

[a] Experiments conducted by the Office of Cereal Investigations of the Bureau of Plant Industry in cooperation with the State agricultural experiment stations.

[b] Damaged by hail.

TABLE IV.—*Summary of milling and baking data from 49 samples of Hard Federation wheat and from 44 of these Hard Federation samples and 44 comparable samples of Marquis grown in one or more of the five years from 1918 to 1922, inclusive* [a]

Descriptive data	All Hard Federation samples	Comparable samples		
		Hard Federation	Marquis	Hard Federation in percentage of Marquis
Number of samples	49	44	44	
Bushel weight	59.0	58.8	58.9	99.8
Crude-protein content of wheat [b] ..... per cent	13.5	13.7	14.0	97.9
Yield of straight flour ..... do	71.7	71.6	73.2	97.8
Yield of shorts ..... do	15.2	15.2	14.1	107.8
Yield of bran ..... do	14.3	14.5	14.9	97.3
Water absorption of flour ..... do	63.3	63.2	60.7	104.1
Volume of loaf cc	2,147	2,152	2,211	97.3
Weight of loaf ..... grams	509	509	503	101.2
Texture of crumb ..... per cent	89.7	89.8	88.9	101.0
Color of crumb ..... do	93.0	93.0	91.7	101.4
Ash in flour ..... do	[c] 47.0	[d] 47.0	[d] 51.0	92.2

[a] Experiments conducted by the Milling Investigations Section, Grain Division, of the Bureau of Agricultural Economics.
[b] (N × 15.7, basis 13.5 per cent moisture).
[c] 48 samples.
[d] 43 samples.

METHODS OF STUDY

The Kota × Hard Federation and reciprocal crosses here studied were made at the request of the writer by Florell (*12*) at Chico, Calif., in May, 1920. The F_1 material, which included 82 plants, was harvested by the writer at Chico, Calif., in June, 1921. The material was divided, one head from each plant being retained in California to furnish seed for sowing at Davis, Calif., and the remainder sent to Washington, D. C., for growing, storage, and distribution to other stations. About 450 seeds were grown in the greenhouse at Arlington Experiment Farm, near Washington, D. C., during the winter of 1921–1922, which furnished F_3 material for growing in the field in 1922. A few crosses were made in the greenhouse for furnishing additional F_1 material.

In the spring of 1922 one head from each of the F_1 hybrid plants grown at Chico was sent from Washington to University Farm, St. Paul, Minn., together with small quantities of F_1 and F_2 seed grown in the greenhouse at Arlington. Seed from all of the remaining heads of the F_1 plants, except one from each which was reserved, were sent to Mandan, N. Dak., together with some of the F_2 seed grown in the greenhouse at Arlington.

There was grown, therefore, F_2 material in 1922 at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak. This distribution of the material seemed desirable from both the economic and investigational viewpoints. It carefully guarded against loss of the material and furnished data for study of genetic and environmental influence at three points rather than one. At Davis, Calif., the seed was fall sown and grown under ideal conditions. At St. Paul, Minn., it was grown under conditions artificially insuring rust infection and at Mandan, N. Dak., under usually droughty conditions. In 1922 and 1923 both rust and drought affected the crop somewhat at Mandan.

The seeds from F_1 plants were carefully spaced at all three points to enable the taking of data on individual F_2 plants. Both parents were seeded similarly in check rows together with Marquis. The F_3 material grown in 1922 was not used to complete the inheritance study of the cross, but for advancing material of the cross one year from which to make selections.

In 1923 F_3 selections were grown at Davis, Calif., Mandan, N. Dak., and St. Paul, Minn. In addition a few F_3 selections were grown at Fargo, N. Dak. At Mandan, N. Dak., there also were grown about seventy-five F_4 selections made there the previous season from seed grown in the greenhouse at Arlington. These selections were grown in single 16-foot nursery rows but with different rates of seeding due to the limited and uniform amounts of seed. No yields were obtained from

this nursery, therefore, but further selections were made from it and the most promising homozygous and rust-resistant rows threshed. The grain from twenty-nine of these has been studied in four quality tests in an early effort to determine the quality of the hybrids in comparison with the parents and Marquis.

INHERITANCE OF CHARACTERS

The cross has furnished material for a study of the inheritance of several valuable and interesting plant characters and grain qualities. These characters which have been studied are inheritance of the awn or its absence, color of the glumes, color of the kernels, date of heading, height of plant, resistance to stem-rust infection, and yield. The grain qualities which thus far have been analyzed in the hybrids are crude protein, for the quantity of the gluten; viscosity, for the quality of the gluten; gasoline color test for the color of the flour, and ash determination for the amount of ash in the flour.

As resistance to black stem rust was one of the principal objects of the research, the material grown at University Farm, St. Paul, was artificially inoculated with stem rust, using nine different specialized (biologic) forms of stem rust common to the spring-wheat area. In addition to the epidemic artificially produced at St. Paul, opportunity to study rust resistance of the material was afforded by the occurrence of natural infection at Mandan, N. Dak.

Important factors concerned with drought resistance were thought to be the presence or absence of awns, earliness of heading, height, and plant productiveness. The color of glumes and kernels apparently have no particular connection with resistance to rust or drought, but were included to complete the study of the principal contrasting parental characters in the cross. The kernel color is of great economic importance. Red-kerneled varieties are demanded in the northern Prairie and Great Plains areas, while white wheats are preferred in the Pacific Coast States.

The study here reported is concerned principally with the development of a hard red spring wheat for the northern Great Plains area. The principal experiments were conducted at the Northern Great Plains Field Station, Mandan, N. Dak., because there both rust and drought may occur with destructive severity. The cross, however, is being used for breeding a common white wheat resistant to rust and drought, for the Pacific Coast States, particularly California, where losses from black stem rust are not infrequent.

DESCRIPTION OF THE F_1

The F_1 plants bore apical awns, varying from 3 to 20 mm. in length. This was different from the Hard Federation parent, which is almost entirely awnless. The glumes of the F_1 plants were brown, but a somewhat lighter brown than those of the Hard Federation parent. The plants which were grown at Chico, Calif., showed a slight infection of both stem rust and leaf rust. They were tall but had the stiff stems of the Hard Federation parent. The kernels were not especially hard, being somewhat softer than those of either of the parents, and showed occasional indication of "yellowberry." Some of the kernels appeared to be much softer than others. The kernels were slightly longer than those of either of the parents. A spike of an F_1 plant is shown in Plate 1, A in comparison with spikes of the parents.

The F_1 plants grown at St. Paul, Minn., in 1922, proved to be susceptible to stem rust.

SEGREGATION OF CHARACTERS IN THE F_2 AND F_3

A study of individual plants for the characters previously mentioned was made on the F_2 material grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., except that yields of the individual plants were not obtained from the California material.

In the F_3 certain plant characters were further studied at Mandan, N. Dak., and Davis, Calif. Only at Mandan were yields of individual plants obtained. Notes on rust resistance were obtained at both St. Paul and Mandan.

A summary of the data recorded on the segregation of characters in F_2 and F_3 is presented.

AWNS AND THEIR ABSENCE

Biffen (*4*) stated that "the beardless condition is a dominant, the bearded a recessive character." Other early workers, particularly Tschermak (*35*) and Spillman (*32*), obtained similar results in the first generation and also showed that in the second generation the awnless and awned plants occur in the monohybrid Mendelian ratio of 3:1.

Inheritance in Kota—Hard Federation Crosses Plate I

A.—Typical spikes of the varieties used as parents in the cross studied, together with the first generation progeny. (1) Kota, (2) F_1 hybrid, and (3) Hard Federation

B.—Spikes of F_2 hybrids representing the 5 classes for expression of the awn character, namely, (1) awnless, (2) apically-awnletted, (3) awnletted, (4) short-awned, and (5) awned

In some cases the ratio of the fully awned to the intermediate and awnless in this generation was 1:2:1.

Saunders (*27*) questioned the idea that the first generation between an awnless and an awned wheat always is awnless and maintained that the character of awns in the F_1 varies with the wheats used.

Howard and Howard (*22*) obtained single-factor results in some crosses, but in others between fully awned and absolutely awnless parents, where the F_1 plants were nearly awnless, they were able to separate the F_2 progeny into five or six classes. When all the awned and awn-tipped classes were grouped as awned the ratio of awned to awnless was 15:1, indicating the presence of two factors. They concluded that for certain crosses two factors, B and T, must be present in a homozygous dominant condition in bearded wheats and that the completely awnless plants should be represented by the double recessive factors.

These researches and later studies by others have proved that awnlessness at least is only partially dominant.

In the present study, in which one parent was awned and the other awnless and the F_1 had apical awnlets from 3 to 20 mm. long, the F_2 plants were distributed into five classes, described as (1) awnless, (2) apically-awnletted, (3) awnletted, (4) short-awned, and (5) awned. Spikes representing these classes are shown in Plate 1, B. Class 1, awnless, normally is entirely without awnlets in the apical part of the spike, although a few awnlets 1 to 2 mm. long may occur at the apex under abnormal conditions. Class 2, apically-awnletted, has awnlets 2 to 20 mm. long at the apex of the spike but rarely extending to the central and basal portions. Class 3, awnletted, has awnlets from 3 to 40 mm. long, the shorter occurring at the base of the spike and the length increasing toward the apex. Class 4, short-awned, has short awns throughout, varying from 15 to 50 mm. long but only about half the length of the normal awns. In Class 5, awned, the awns vary from 30 to 80 mm. in length. These five divisions of the material appear, after a careful study, to be rather definite. The data obtained are summarized in Table V. Sixty-eight F_1 families were included in the study of these characters.

The inheritance of the awn or its absence in the Kota-Hard Federation and reciprocal crosses is shown in Table V to be very similar. There is no important or consistent evidence of maternal influence.

Attempting an interpretation of the F_2 results, it is simplest to group as awnless, the three classes (1) awnless, (2) apically-awnletted, and (3) awnletted; and as awned, the two classes (4) short-awned, and (5) awned. The total results are then as follows:

	Awnless, classes 1, 2, 3	Awned, classes 4, 5
Obtained	3,362	1,089
Expected on 3:1 ratio	3,338	1,113
Deviation	24	24
Probable error *		19.5

* Probable errors for numbers of individuals given here and elsewhere in this paper were obtained from tables of probable errors of Mendelian ratios, prepared in the Department of Plant Breeding, Cornell University, Ithaca, N. Y., from the formula $0.6744898\sqrt{npq}$ in which n is the total number of individuals and p and q the numbers corresponding to the ratios concerned.

The deviation of only 24 ± 19.5 shows a very close fit. The presence of one principal genetic factor is thus indicated. It is obvious, however, that this is not a certain or complete explanation.

The F_2 material, in general, contained many more awned or awnless plants than would be expected on a 2-factor hypothesis. Thus the necessity for growing and examining the F_3 progeny to obtain further information was very apparent.

A further study on the inheritance of the awn was made on F_3 material grown at Mandan, N. Dak., and Davis, Calif., in 1923. As many as 434 selections, which were mostly rust-resistant red wheats, were grown at Mandan. Of these 291 were from F_2 material grown there and 143 were from the F_2 material grown at St. Paul, Minn., in 1922. At Davis, there were grown 197 selections which were both red and white wheats from F_2 material grown there the previous year, although they included a few white-kerneled, rust-resistant selections from St. Paul. The data obtained from F_3 material are summarized in Table VI.

TABLE V.—*Segregation of 4,451 F_2 plants of Kota×Hard Federation and reciprocal crosses into five classes for awns or their absence, grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and cross	Number of F_1 families	Number and per cent of F_2 plants in classes					
		Awnless	Apically-awnletted	Awnletted	Short-awned	Awned	Total of all classes
DAVIS, CALIF.							
Kota×Hard Federation:							
Numbers	11	66	144	75	38	26	349
Percentages		18.9	41.3	21.5	10.9	7.4	100
Hard Federation×Kota:							
Numbers	19	78	287	154	113	46	678
Percentages		11.5	42.3	22.7	16.7	6.8	100
Total:							
Numbers	30	144	431	229	151	72	1,027
Percentages		14.0	42.0	22.3	14.7	7.0	100
ST. PAUL, MINN.							
Kota×Hard Federation:							
Numbers	20	49	201	233	79	94	656
Percentages		7.5	30.7	35.5	12.0	14.3	100
Hard Federation×Kota:							
Numbers	40	66	455	221	130	94	966
Percentages		6.8	47.1	22.9	13.5	9.7	100
Total:							
Numbers	60	115	656	454	209	188	1,622
Percentages		7.1	40.4	28.0	12.9	11.6	100
MANDAN, N. DAK.							
Kota×Hard Federation:							
Numbers	12	64	391	386	149	156	1,146
Percentages		5.6	34.1	33.7	13.0	13.6	100
Hard Federation×Kota:							
Numbers	15	59	295	138	71	93	656
Percentages		9.0	45.0	21.0	10.8	14.2	100
Total:							
Numbers	27	123	686	524	220	249	1,802
Percentages		6.8	38.1	29.1	12.2	13.8	100
Grand total:							
Numbers	68	382	1,773	1,207	580	509	4,451
Percentages		8.6	39.9	27.1	13.0	11.4	100

TABLE VI.—*Segregation of 16,144 F_3 plants of the Kota-Hard Federation and reciprocal crosses for awn classes at Mandan, N. Dak., and Davis, Calif., in 1923*

F_2 classes and breeding behavior in the F_3	Segregation of F_2 families and F_3 plants								
	Mandan, N. Dak.								
	F_2 families		Per cent of F_2[a]	Classes of F_3 plants					Total
	Number of plants	Per cent of class		1	2	3	4	5	
Awnless, 1:									
1	1	2.9	0.2	16					16
1 and 2	20	57.1	3.9	288	176				464
1 to 3	7	20.0	1.4	57	72	25			154
1 to 4	7	20.0	1.4	84	46	16	16		162
Class total	35	100.0	6.9	445	294	41	16		796
Apically-awnletted, 2:									
2	9	6.0	2.3		193				193
2 and 3	41	27.3	10.4		691	238			929
2 to 4	28	18.6	7.1		429	98	112		639
2 to 5	26	17.3	6.6		267	149	65	76	557
1 and 2	4	2.7	1.0	27	68				95
1 to 3	16	10.7	4.1	55	224	88			367
1 to 4	19	12.7	4.8	73	236	63	67		439
1 to 5	7	4.7	1.8	20	81	26	22	29	178
Class total	150	100.0	38.1	175	2,189	662	266	105	3,397
Awnletted, 3:									
3	9	8.1	2.4			224			224
3 to 5	19	17.1	5.0			352	81	31	464
2 to 5	64	57.7	16.7		427	881	211	150	1,669
1 to 5	12	10.8	3.1	25	84	126	45	27	307
1 to 4	6	5.4	1.6	14	35	81	17		147
1 to 3	1	.9	.3	4	16	8			28
Class total	111	100.0	29.1	43	562	1,672	354	208	2,839
Short-awned, 4:									
4	2	4.8	.6				43		43
4 to 5									
3 to 5	18	42.8	5.2			198	169	55	422
3 to 4	16	38.1	4.6			194	179		373
2 to 5	4	9.5	1.2		13	42	40	17	112
2 to 4	2	4.8	.6		17	22	6		45
Class total	42	100.0	12.2		30	456	437	72	995
Awned, 5:									
5	23	24.0	3.3					560	560
4 and 5	18	18.7	2.6				92	336	428
3 to 5	52	54.2	7.5			160	294	801	1,255
2 to 5	3	3.1	.4		13	26	7	24	70
Class total	96	100.0	13.8		13	186	393	1,721	2,313
Grand total	434	500.0	100.1	663	3,088	3,017	1,466	2,106	10,340

TABLE VI.—*Segregation of 16,144 F_3 plants of the Kota-Hard Federation and reciprocal crosses for awn classes at Mandan, N. Dak., and Davis, Calif., in 1923*—Continued

F_2 classes and breeding behavior in the F_3	Segregation of F_2 families and F_3 plants—Continued								
	Davis, Calif.								
	F_2 families		Per cent of F_2[a]	Classes of F_3 plants					Total
	Number of plants	Per cent of class		1	2	3	4	5	
Awnless, 1:									
1	15	29.4	4.1	392					392
1 and 2	12	23.5	3.3	227	116				343
1 to 3	21	41.2	5.8	324	215	88			627
1 to 4	3	5.9	.8	36	19	19	8		82
Class total	51	100.0	14.0	979	350	107	8		1,444
Apically-awnletted, 2:									
2	1	2.0	.8		31				31
2 and 3	6	12.0	5.0		139	57			196
2 to 4	4	8.0	3.4		51	48	23		122
2 to 5	2	4.0	1.7		32	19	7	12	70
1 and 2	4	8.0	3.4	69	58				127
1 to 3	14	28.0	11.8	126	275	87			488
1 to 4	5	10.0	4.2	46	93	30	19		188
1 to 5	14	28.0	11.8	58	190	100	54	44	446
Class total	50	100.0	42.1	299	869	341	103	56	1,668
Awnletted, 3:									
3									
3 to 5									
2 to 5	7	53.8	12.0		80	80	49	33	242
1 to 5	4	30.8	6.9	26	65	31	20	13	155
1 to 4	1	7.7	1.7	3	15	8	3		29
1 to 3	1	7.7	1.7	6	26	6			38
Class total	13	100.0	22.3	35	186	125	72	46	464
Short-awned, 4:									
4									
4 to 5	1	5.3	.8				6	3	9
3 to 5	9	47.3	7.0			49	104	66	219
3 to 4	1	5.3	.8			12	13		25
2 to 5	7	36.8	5.4		18	64	85	35	202
2 to 4	1	5.3	.8		15	31	3		49
Class total	19	100.0	14.8		33	156	211	104	504
Awned, 5:									
5	49	76.6	5.4					1,296	1,296
4 and 5	4	6.2	.4				15	114	129
3 to 5	6	9.4	.7			30	50	68	148
2 to 5	5	7.8	.5		14	29	57	51	151
Class total	64	100.0	7.0		14	59	122	1,529	1,724
Grand total	197	500.0	100.2	1,313	1,452	788	516	1,735	5,804

[a] Weighted in proportion to percentage of F_2 plants in each class of total for same station in 1922 given in Table V.

The F_1, which was apically-awnletted, approached more closely to the awnless than the awned parent. If the data can be interpreted on a 1-factor basis it is necessary to look for the 25 per cent of recessives among those having more awn development than F_1. In F_2, classes 4 and 5 combined include 26 per cent at Mandan, 24.5 per cent at St. Paul, and 21.7 per cent at Davis. The total number of plants, constituting 24.4 per cent, was shown to be not significantly different from the 3:1 ratio and suggests that the awned and short-awned classes represent the recessive type.

In F_3, however, the awned or short-awned classes did not breed true within the limits of classes 4 and 5 combined, either at Mandan or at Davis. At both points only about 6.5 per cent of the whole F_2 bred true to these limits. Thus the simple hypothesis that classes 4 and 5 are due to a single recessive factor is untenable.

It may be assumed that those plants recessive with respect to the primary factor, if any, are distributed among classes 3, 4, and 5. As these classes make up 55.1 per cent of the F_2 population at Mandan and 44 per cent of that at Davis, it is necessary to assume that a large part of classes 3, 4, and perhaps even 5 (which does not wholly breed true within the limits of classes 3 to 5) exceed the F_1 in amount of awn because of some other factor than the one being considered as primary. Only a part of these classes (3, 4, 5) breed true to their wide range. At Mandan, 31.2 per cent of the F_2 was composed of such plants (classes 3, 4, and 5 producing only 3, 4, and 5) but only 15.1 per cent at Davis. Evidently it is possible to find a recessive class in the Mandan group data, but the Davis group data indicate that some of these classes recessive in the most inportant factor must have been as low as class 2. It is very clear that there is no one outstanding recessive factor responsible for the awned condition.

Looking at the other end of the F_2 series for the homozygous dominant class, it is shown in Tables V and VI that while 44.9 per cent at Mandan and 56 per cent at Davis were of classes 1 and 2, there were only 7.4 per cent at Mandan, and 11.6 per cent at Davis, of classes 1 and 2 of the F_2 population which produced only classes 1 and 2. Thus with reference to the most important factor homozygotes must be looked for in class 3 as well as in classes 1 and 2. Twenty-six per cent of the F_2 generation at Mandan and 35.9 per cent at Davis, were of classes 1, 2, and 3 producing only 1, 2, and 3. These can include all homozygous dominants. Thus it is possible that there may be a dominant factor present in classes 1 to 3 and a recessive factor present in classes from 3 to 5 at Mandan and in classes 2 to 5 at Davis, with heterozygotes appearing in classes from 1 to 4 and perhaps even 5.

To account for this wide range and necessary overlap of the classes containing the homozygous dominant or recessive for the most important factor, it is necessary to assume the existence of a second factor, or a group of factors, nearly if not fully as important as the first.

On the hypothesis that there are two factors of equal importance, it will be necessary to find 6.25 per cent of the F_2 strains at each extreme breeding true. Only 3. 3 per cent of the Mandan F_2 were of class 5 breeding true to 5, and 5.4 per cent of the Davis F_2 were of that sort. It thus is necessary to suppose that some of the 2-factor recessives were in class 4. At the other extreme only 0.2 per cent of the Mandan F_2 were of class 1 breeding true to 1, and 4.1 per cent of the Davis F_2 were of that sort. Thus some 2-factor homozygous dominants must be looked for in class 2. There were 6.4 per cent of F_2 classes 1 and 2 which produced only 1 and 2 at Mandan, and 11.6 per cent in the Davis F_2. At the other extreme there were 6.5 per cent of Mandan F_2 and 6.6 per cent of Davis F_2 of classes 4 and 5 producing only 4 and 5. The 2-factor homozygous dominant and recessive (expectation 6.25 per cent) thus can be found in classes 1 to 2 and 4 to 5, respectively, but additional factors are necessary to account for the variation remaining.

To have arbitrarily assumed the awnless to be the recessive class, as Howard and Howard (*22*) had done in their 2-factor hypothesis and then grouped the remaining classes as awned, 6.25 per cent of the awnless plants should have bred true. As only 0.2 per cent of class 1 bred true for class 1 at Mandan, and 4.1 per cent at Davis, it is apparent that their findings could not apply to this cross. The writer, therefore, assumes that, for the Kota-Hard Federation and reciprocal crosses here studied, two factors at least must be present in a dominant condition in awnless strains, and that the awned plants should be represented at least by double recessive factors. This is a conclusion opposite to that of Howard and Howard.

The 2-factor hypothesis here advanced does not entirely explain the

inheritance of awns in this cross. Complete homozygosity for awned or awnless strains apparently is due to multiple factors.

EFFECT OF AWNS ON YIELD.—The physiological effect of the awn on yield is a point of considerable economic importance. Grantham (*17*) found that awned wheats outyielded awnless on the average of many varieties. Hayes (*19*) has summarized the important papers on this subject, and after a study of awnless, tip-awned, and bearded strains from Marquis-Preston crosses states:

> The awn of wheat is, therefore, an important organ, and the present tendency to breed only awnless wheat should not be adopted in entirety without further experimental studies.

Most of the physiological studies have been with barley, the most recent being that of Harlan and Anthony (*18*) who found that the elimination of the awns resulted not only in lower yields, but in increased tendency toward shattering as well. Perlitius (*26*) worked with wheat as well as barley. He made transpiration studies with both winter and spring wheats, using (1) awned, (2) awns removed, and (3) awnless lots. He concluded:

> The awns of the spike are important for transpiration. This transpiration is nearly half that of the total transpiration of the head when awned heads of wheat and heads with awns removed are compared.

He also stated:

> The awn has an important influence on the volume and weight of the kernel [and] a marked effect on kernel quality which exhibited itself chiefly in an increase in starch content in awned sorts.

Schmid (*28*) had previously shown, after careful experiments, that awnless varieties have higher gluten content than awned. He also stated that—

> the physiological service of the awn supplies a not unimportant work for the normal building of the fruit. The amount of its importance stands in direct relation to the size of the awn.

In the present study the mean yields of F_2 plants of each awn class and their probable errors have been obtained from two sources. The data are given in Table VII.

The F_2 data, especially those from Mandan, N. Dak., show that there is a direct relation between the awn length and the yield. The data from St. Paul are not as consistent as those from Mandan, although the difference between the extreme and the parent classes is greater. The difference between the awnless and awned classes at St. Paul is 15 per cent, or 0.56 ± 0.23 grams. This difference is 2.43 times its probable error and represents odds of about 9:1. At Mandan the difference between the same classes is 11 per cent or 0.44 ± 0.18 grams. This difference is 2.44 times its probable error, also indicating odds of about 9:1. While these differences can not be definitely said to be significant, the awns apparently are of some importance under droughty as well as humid conditions.

In 1923 nine hundred F_3 plants grown at Mandan, N. Dak., were threshed and the yields of the five awn classes averaged. These data are given in Table VIII.

TABLE VII.—*Mean yield and its probable error of five awn classes of F_2 plants of Kota-Hard Federation crosses grown at St. Paul, Minn., and Mandan, N. Dak. in 1922*

Locality and class	Number of plants	Yield in grams, mean and probable error
ST. PAUL, MINN.		
Awnless	45	3.70±0.17
Apically-awnletted	171	3.67± .11
Awnletted	200	4.15± .12
Short-awned	68	3.85± .18
Awned	78	4.26± .15
Total and average	562	3.95± .06
MANDAN, N. DAK.		
Awnless	63	3.91± .15
Apically-awnletted	389	4.11± .08
Awnletted	387	4.13± .06
Short-awned	148	4.16± .09
Awned	156	4.35± .10
Total and average	1,143	4.06± .03

TABLE VIII.—*Mean yield and its probable error for five awn classes of F_3 plants of Kota-Hard Federation crosses grown at Mandan, N. Dak., in 1923*

Class	Number of plants	Yield in grams, mean and probable error
Awnless	126	2.79±0.06
Apically-awnletted	403	3.02± .04
Awnletted	111	3.02± .07
Short-awned	73	3.06± .08
Awned	187	3.30± .06
Total and average	900	3.05± .03

These further data show a similar definite relationship between the awn length and yield. The difference between the extreme classes in this latter case is 18 per cent or 0.51 ± 0.08 gram. As this difference is over six times its probable error it certainly may be considered significant. Many factors are associated with the complex character designated yield, and it appears that the factors for the development of awns are among those involved.

COLOR OF THE GLUMES

Glume colors are variously classed as white, yellowish, brown or red. Two classes usually are used, namely, white and brown. Those classed as white may vary from nearly white to yellowish and those classed as brown may show various shades of brown to a brownish red. Biffen (4) found brown glume color dominant to white in the single 3 to 1 ratio. No other ratio is known to have been reported by other workers in crosses between common wheats.

The present study includes F_2 data on the segregation for color of the glumes at the three points, Davis, Calif., St. Paul, Minn., and Mandan, N. Dak. The results obtained are given in Table IX.

TABLE IX.—*Segregation of 4,442 F_2 plants of the Kota-Hard Federation and reciprocal crosses into two classes for color of glumes, when grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and cross	Number of F_1 families	Number and percentage of F_2 plants having—				Total number	Deviation from 3:1 ratio	Probable error
		Brown glumes		White glumes				
		Number	Per cent	Number	Per cent			
DAVIS, CALIF.								
Kota×Hard Federation	11	245	70.0	105	30.0	350	18	5.46
Hard Federation×Kota	19	490	72.1	190	27.9	680	20	7.62
Total	30	735	71.4	295	28.6	1,030	38	9.38
ST. PAUL, MINN.								
Kota×Hard Federation	20	376	57.7	276	42.3	652	113	7.46
Hard Federation×Kota	40	665	69.3	294	30.7	959	54	9.04
Total	60	1,041	64.6	570	35.4	1,611	167	11.73
MANDAN, N. DAK.								
Kota×Hard Federation	12	789	68.9	356	31.1	1,145	70	9.88
Hard Federation×Kota	15	502	76.5	154	23.5	656	10	7.48
Total	27	1,291	71.7	510	28.3	1,801	60	12.40
ALL THREE STATIONS								
Kota×Hard Federation	23	1,410	65.7	737	34.3	2,147	200	13.54
Hard Federation×Kota	45	1,657	72.2	638	27.8	2,295	64	13.99
Grand total	68	3,067	69.0	1,375	31.0	4,442	264	19.48

With this character there is evidence of slight maternal influence on the reciprocal crosses as there is a consistant difference in the percentage at all three points. The percentages of white-glumed plants are the greater when Kota is used as the female parent and the percentages of brown-glumed plants are greater with Hard Federation as the female parent.

The totals of white-glumed plants in the reciprocal crosses at each of the three stations in the grand total show that there resulted a considerably larger number of plants having white glumes than would be expected on a 3:1 basis in general, the ratio being nearer 2:1 than 3:1. A close fit to the expected 3:1 ratio was obtained for the Hard Federation × Kota cross at Davis and Mandan, where the deviations were 20 ± 7.62 and 10 ± 7.48, respectively. At St. Paul, the results from this cross also were much nearer a good fit than the reciprocal, although significantly different from the expected ratio, which is true also for the total. It is important, however, that where Hard Federation, which has brown glumes and the dominant color, was used as the female parent, a close fit was obtained under two of the three different sets of conditions.

It will be recalled that brown glumes in the F_1 were a somewhat lighter brown than that of Hard Federation. Considerable variation was apparent in the F_2 regarding glumes classed as brown or white. The glumes of Kota are yellowish rather than white, but there appeared to be some white-glumed plants in the F_2 progeny. The differences, however, were not contrasting enough to permit any more definite grouping, even with the material from Davis, Calif., where growing conditions were ideal and the colors almost perfectly developed. At Mandan and more particularly at St. Paul, summer rains and damage by rust made separations on the two colors difficult. The study was continued in the F_3 at Mandan and Davis and the data obtained are given in Table X.

TABLE X.—*Segregation of 15,946 F_3 plants of the Kota-Hard Federation and reciprocal crosses into two classes for color of glumes, when grown at Mandan, N. Dak., and Davis, Calif., in 1923*

Source, F_2 classes, and segregation in the F_3	F_2 families		F_3 plants		
	Number	Percentage of class	Percentage of F_2[a]	Glumes	
				Brown	White
MANDAN, N. DAK.					
Glumes white:					
White	126	81.8	23.1	----------	2,918
White and brown	28	18.2	5.2	280	360
Total	154	100.0	28.3	280	3,278
Glumes brown:					
Brown	72	25.8	18.5	1,704	----------
Brown and white	207	74.2	53.2	3,300	1,657
Total	279	100.0	71.7	5,004	1,657
Mandan total	433	200.0	100.0	5,284	4,935
DAVIS, CALIF.					
Glumes white:					
White	75	89.3	25.5	----------	2,118
White and brown	9	10.7	3.1	156	57
Total	84	100.0	28.6	156	2,175
Glumes brown:					
Brown	45	39.8	28.4	1,350	----------
Brown and white	68	60.2	43.0	1,488	558
Total	113	100.0	71.4	2,838	558
Davis total	197	200.0	100.0	2,994	2,733

[a] Weighted in proportion to percentage of F_2 plants in each class of total for same station in 1922, given in Table IX.

The F_3 results show that some of the plants classed as white segregated for white and brown. The percentages of these were larger at Mandan than at Davis. Correcting the F_2 Davis results on the basis of the F_3 plants grown at that station, the following results are obtained:

	Brown	White
Obtained (uncorrected)	735	295
Corrected on basis of F_3	767	263
Expected on 3:1 ratio	772	258
Deviation	5	5
Probable error	9.38	

The deviation of 5 ± 9.38 shows a very close fit.

A similar correction of the Mandan F_2 results on the basis of the F_3 plants grown there gives the following:

	Brown	White
Obtained (uncorrected)	1,291	510
Corrected on basis of F_3	1,384	417
Expected on 3:1 ratio	1,351	450
Deviation	33	33
Probable error	12.4	

This deviation of 33 ± 12.4 is not significantly different from the expected ratio as it is less than three times the probable error.

At St. Paul the F_2 plants were shown to have a greater percentage of white glumed plants than occurred at either Davis or Mandan. The Kota-Hard Federation cross particularly segregated in that manner, due possibly to maternal influence, although the environmental conditions at St. Paul were less favorable than at the other points for developing and maintaining natural glume colors. It is quite probable, therefore, that there was a greater error in the classification of the St. Paul material, which, together with the possible maternal influence, may account for the differences.

Of the 28 F_2 white-glumed families at Mandan which segregated for white and brown glumes, 19 produced more white-glumed plants than brown. The average for the 28 families was 13 white to 10 brown. There were also 7 additional families included among the true-breeding white which produced 1 to 4 questionable brown-glumed plants. The occurrence of these questionable plants or of more white-glumed plants than brown can not be satisfactorily explained and may be due to natural crossing. At Davis the 9 families classed as white-glumed in F_2 and which broke up into brown-glumed and white-glumed in F_3 segregated in the expected 3:1 ratio, the deviation being 4 ± 4.26. There were no families which had more white-glumed plants than brown, although one family had an equal number of each.

The number of F_2 families of the brown-glumed class at Mandan separated into homozygous and heterozygous families in the F_3 closer to a 1:3 ratio than to the expected 1:2. At Davis, however, a close agreement to the 1:2 ratio was obtained, the deviation being 7 ± 3.24, which is not significantly different from the expected.

Of the heterozygous brown-glumed F_2 families at Mandan the F_3 plants segregated in a ratio very close to 1:2 rather than 1:3. This is very similar to the F_2 results and is what would be expected to reoccur. At Davis, although the ratio is nearer the 1:3, the deviation is 46 ± 13.24, which also is significantly different.

In spite of the several unexpected segregations and ratios obtained in both F_2 and F_3 there is no reliable evidence of more than a single genetic factor involved in the color of glumes in this cross. The frequent lack of a significant fit to the 3:1 ratio apparently is due to environment, but may be due to natural crossing and possibly maternal influence.

COLOR OF THE KERNEL

The inheritance of kernel color has been explained by one, two, and three Mendelian factors. Biffen (*4*) found that red was dominant to white in F_1 and segregated in a 3:1 ratio in F_2. Nilsson-Ehle (*24*) was the first to report crosses which in F_2 gave 15:1 and 63:1 ratios of red-kerneled and white-kerneled plants, proving the presence of two and three factors. Howard and Howard (*22*) of India, and Gaines (*15*) in Washington have since obtained similar ratios.

In the cross here reported red proved dominant as usual in F_1, and the data on segregation in F_2 are given in Table XI.

TABLE XI.—*Segregation of 4,432 F_2 plants of the Kota-Hard Federation and reciprocal crosses into two classes for color of kernels, when grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and cross	Number of F_1 plants	Number and percentage of F_2 plants					Deviation from 15:1	Probable error
		Red kernels		White kernels		Total number		
DAVIS, CALIF.			*Per cent*		*Per cent*			
Kota×Hard Federation	11	325	92.9	25	7.1	350	3	3.05
Hard Federation×Kota	19	643	94.6	37	5.4	680	5	4.26
Total	30	968	94.0	62	6.0	1,030	2	5.24
ST. PAUL, MINN.								
Kota×Hard Federation	20	615	94.3	37	5.7	652	4	4.17
Hard Federation×Kota	40	922	96.1	37	3.9	959	23	5.06
Total	60	1,537	95.4	74	4.6	1,611	27	6.52
MANDAN, N. DAK.								
Kota×Hard Federation	12	1,085	94.9	58	5.1	1,143	13	5.53
Hard Federation×Kota	15	626	96.6	22	3.4	648	19	4.16
Total	27	1,711	95.5	80	4.5	1,791	32	6.88
ALL THREE STATIONS								
Kota×Hard Federation	23	2,025	94.4	120	5.6	2,145	14	7.59
Hard Federation×Kota	45	2,191	95.8	96	4.2	2,287	47	7.79
Grand total	68	4,216	95.1	216	4.9	4,432	61	10.86

The data in Table XI show a fairly good agreement to a 2-factor or 15:1 ratio. For this ratio 93.75 per cent should be red-kerneled and 6.25 per cent white. The grand total shows 95.1 per cent red and 4.9 per cent white or a deviation from the 15:1 ratio of 59 ± 10.86, and, therefore, is not a close fit. A more detailed study of the data shows that in some cases there were no significant differences.

There again is evidence of slight maternal influence on this character in the reciprocal crosses, as there is a consistant difference in the percentages at all three points. The numbers and percentages for the Kota×Hard Federation cross show a close fit to the two-factor hypothesis at each of the three localities and in the total for all. The reciprocal cross, however, in which Hard Federation is the female parent and which has the white or recessive type of kernel, shows a close fit at only one of the three localities, Davis, Calif., and not in the total for all.

A more certain separation of the kernels into the red and white classes was possible with the Davis, Calif., material than with that from St. Paul, Minn., and Mandan, N. Dak. Environmental conditions could account for the close fit which was obtained from the total material grown at Davis and for the significantly different results for the total material at St. Paul and Mandan.

It would appear therefore that two Mendelian factors are involved in kernel color in this cross, and that the lack of a significantly close fit to the 15:1 ratio in the reciprocal or Hard Federation×Kota cross and in the grand total is due to environmental conditions and possible slight maternal influence. When Kota, which has the dominant red kernels, was used as the female parent, a significantly close fit was obtained under all three different conditions.

In order further to determine the factors involved, F_3 material of the 1923 crop from Davis, Calif., was studied. The data obtained are given in Table XII.

TABLE XII.—*Segregation of 5,737 F_3 plants of the Kota-Hard Federation and reciprocal cross into two classes for color of kernel, when grown at Davis, Calif., in 1923*

F_2 classes and segregation in the F_3	Davis, Calif.						
	F_2 families		F_3 plants				
	Numbers	Percentage of class	Percentage of F_2	Kernels		Deviation from ratio indicated	Probable error
				Red	White		
Kernels white:							
White	80	100.0	6.0	----------	2,495	----------	----------
Total	80	100.0	6.0	----------	2,495	----------	----------
Kernels red:							
Red	55	47.0	44.2	1,501	----------	----------	----------
Red and white—							
3:1 ratio	37	31.6	29.7	821	254	15	9.59
15:1 ratio	25	21.4	20.1	623	43	1	4.21
Total	117	100.0	94.0	2,945	297	----------	----------
Grand total	197	200.0	100.0	2,945	2,792	----------	----------

The data show that the white-kerneled strains bred true to that color. The red-kerneled strains bred true or segregated into red and white in either the 3:1 or 15:1 ratios. From the 37 F_2 families which segregated according to the 3:1 ratio there were 821 red-kerneled plants to 254 white in F_3, a deviation from the expected of 15 ± 9.59. Of the 666 F_3 plants which segregated according to the 15:1 ratio the deviation from expected was 1 ± 4.02, which indicates an unusually close fit.

Of the F_2 red-kerneled strains, seven-fifteenths, or 46.7 per cent, should have bred true and four-fifteenths, or 26.7 per cent, should have segregated in both the 3:1 and the 15:1 ratios. As shown in Table XII, the percentages obtained were very close to the expected.

DATE OF HEADING

Earliness often is an important economic factor in successful spring-wheat production in the dry sections of the United States. The date of heading is thought by the writer to be the best note to use in a study of earliness under drought and rust conditions. It is more reliable and shows a greater range of variation than the date of ripening where adverse environmental conditions affect the wheat crop near maturity.

Farrer (*11*) found earliness in wheat hybrids to be intermediate between the parents and that there was no difference in the reciprocal of a cross. Biffen (*4*) in an interspecific cross between Polish, an early wheat, and Rivet, a late wheat, concluded that earliness was dominant. Freeman (*14*), in a cross between durum and common wheat, found in F_2 and F_3 that the average date of heading, while intermediate, was nearer that of the late parent, indicating that lateness is at least partially dominant. Thompson (*34*) made numerous crosses between eight varieties of wheat ranging from very early to late. In the F_1 nearly all crosses ripened near the mean of the later parent. In the F_2 the great majority of plants were intermediate between the parents, indicating blending. The apparent dominance of lateness in F_1 could be explained only as due to heterosis or hybrid vigor. The further results of individual crosses were explained on the "multiple determiner hypothesis of blending." Bryan and Pressley (*5*), in a cross between Sonora and Turkey, found the F_1 intermediate in time of heading between the parents and the F_2 majority inclined toward the late parent. Florell (*13*) in a Sunset-Marquis cross concluded earliness to be dominant from F_2 "in the proportion of 3.11 to 0.89, indicating one allelomorphic pair of factors, with possibly a number of minor modifying factors."

TABLE XIII.—*Segregation of 2,487 F_2 plants of the Kota-Hard Federation or reciprocal crosses, in comparison with parents, into two-day classes for date of heading, when grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and class by dates of heading	Number and percentage of plants on date of heading							
	F_2 plants				Hard Federation		Kota	
	Kota × Hard Fed.	Hard Fed. × Kota	Total	Percentage	Number	Percentage	Number	Percentage
DAVIS, CALIF.								
Apr. 20					1	2.5		
22		4	4	0.5	8	20.0		
24	5	10	15	1.7	15	37.5		
26	23	48	71	8.1	13	32.5		
28	62	169	231	26.3	2	5.0		
30	113	216	329	37.5	1	2.5		
May 2	56	75	131	14.9				
4	31	45	76	8.7				
6	8	10	18	2.1			2	5.1
8	1	2	3	.3			21	53.8
10							12	30.8
12							2	5.1
14							2	5.1
Total	299	579	878	100.1	40	100.0	39	99.9
ST. PAUL, MINN.								
June 18	286			52.9	15	48.4		
20	109			20.3	9	29.0	4	11.4
22	70			12.9	4	12.9	15	42.8
24	34			6.3			12	34.3
26	30			5.5	3	9.7	3	8.6
28	12			2.2			1	2.9
Total	541			100.1	31	100.0	35	100.0
MANDAN, N. DAK.								
June 26	4			.4				
28	205			19.2	3	4.4		
30	253			23.7	28	41.2		
July 2	154			14.4	19	27.9		
4	197			18.4	8	11.8	1	1.2
6	154			14.4	9	13.2	32	39.0
8	65			6.1	1	1.5	28	34.1
10	24			2.3			17	20.7
12	7			.7			3	3.7
14	5			.5			1	1.2
Total	1,068			100.1	68	100.0	82	99.9

TABLE XIV.—*Average date of heading of F_2 hybrids and of the parents, Kota and Hard Federation, grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922*

Station	F_2 hybrid		Kota		Hard Federation	
	Number of plants	Date	Number of plants	Date	Number of plants	Date
Davis, Calif.	828	Apr. 30	39	May 9	40	Apr. 24
St. Paul, Minn.	541	June 20	35	June 22	31	June 19
Mandan, N. Dak.	1,068	July 2	82	July 10	68	July 2

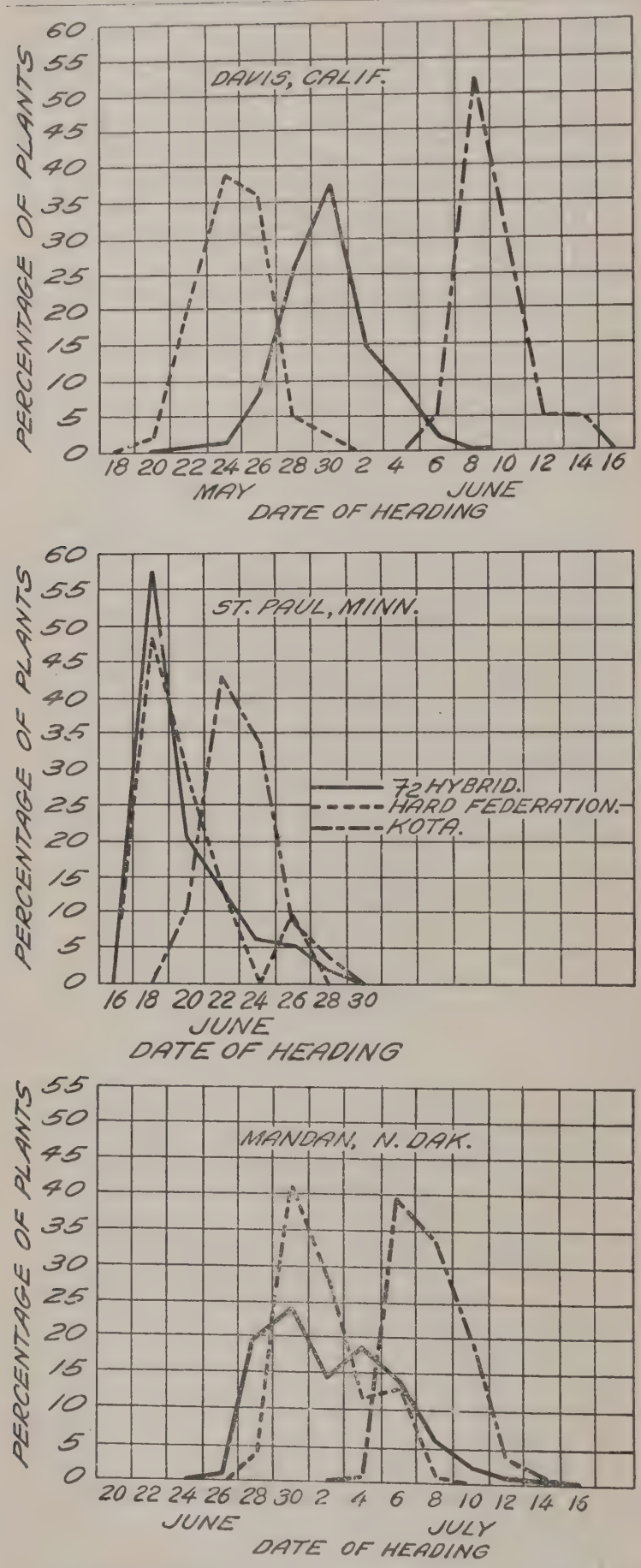
DAVIS, CALIF.
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55 60
18 20 22 24 26 28 30 2 4 6 8 10 12 14 16
MAY
JUNE
DATE OF HEADING
ST. PAUL, MINN.
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55 60
72 HYBRID.
HARD FEDERATION.
KOTA.
16 18 20 22 24 26 28 30
JUNE
DATE OF HEADING
MANDAN, N. DAK.
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55
20 22 24 26 28 30 2 4 6 8 10 12 14 16
JUNE
JULY
DATE OF HEADING

FIG. 1.—Frequency distribution, by dates of heading, of F_2 hybrids and of the Kota and Hard Federation parents at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922

In the present study comparisons of F_1 with parents are not available, but F_2 data compared with those from parent plants are available from three sources. Only at Davis, Calif., were reciprocal crosses studied for this character. The frequency distribution obtained is given in Table XIII and shown graphically in Figure 1. In the background of Plate 2 are shown the labeled plants growing at Davis, Calif. The dates of heading were obtained by tagging the plants individually on the day the first head emerged from the sheath. The average dates of heading for the F_2 hybrids and for the parents are given in Table XIV.

At Davis, Calif., the crop was fall sown and during the ideal spring conditions the dates of heading of the parents extended over a period of 26 days. The F_2 hybrids headed there during 18 of these days, as shown in Table XIII and in the upper portion of Figure 1. Earliness in the F_2 progeny appears partially dominant as the mode is nearer that of the early parent than of the late one. The reciprocal crosses at Davis show slight maternal influence. The mean dates together with their probable errors are as follows:

Kota × Hard Federation, April	29. 65 ± . 10
Hard Federation × Kota, April	29. 13 ± . 07
Difference	. 52 ± . 12

This difference of 0.52 ± 0.12 days, while small, is significant as it is 4.3 times its probable error, with odds of 267: 1 against the occurrence of such a difference being due to chance alone. With earliness dominant and present in the female parent, the slight maternal influence is exerted in the same manner as in the case of color of glumes and color of kernel.

Inheritance in Kota—Hard Federation Crosses

Plate 2

Measuring the height of plant of F_2 hybrids of the Kota-Hard Federation cross at Davis, Calif., in 1922

Further evidence that earliness is the dominant condition in this cross is shown by the results from St. Paul and Mandan in Table XIII and the central and lower portions, respectively, of Figure 1. At these points the material was spring sown and the heading period was shorter. At St. Paul heading started suddenly and the data obtained do not show normal variability. However, earliness appears dominant. At Mandan excellent data were obtained, which show the F_2 progeny to be much more variable than either of the parents and the mode and the mean to be the same as that of the early Hard Federation parent. The bimodal curve for the F_2 progeny and Hard Federation at Mandan was due principally to a rain which occurred on the 2d of July. It does not seem desirable to attempt a factor interpretation.

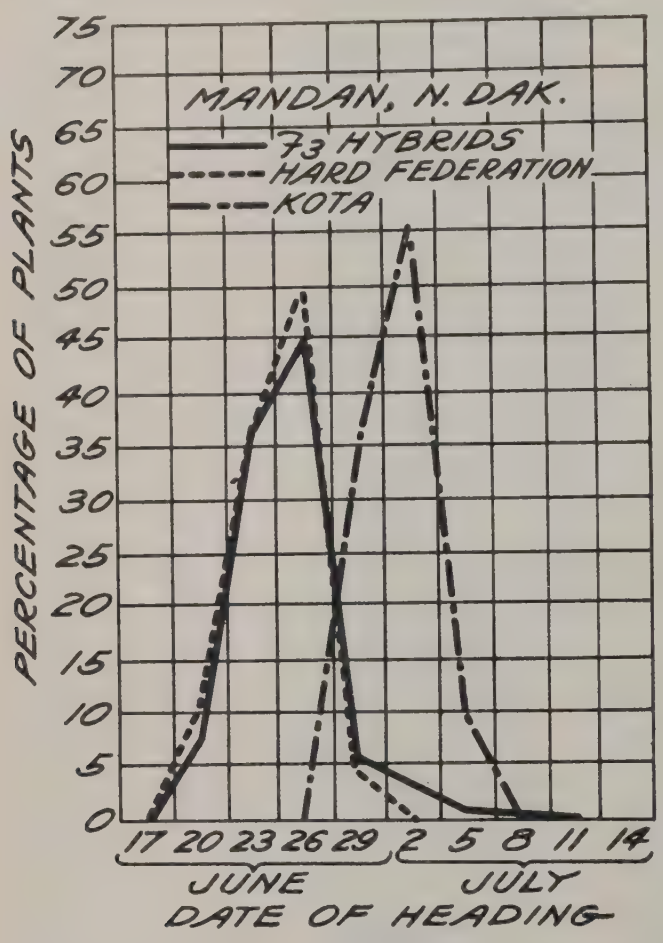

FIG. 2.—Frequency distribution, by dates of heading, of F_3 selections and of the Kota and Hard Federation parents at Mandan, N. Dak., in 1923

In 1923 at Mandan, N. Dak., 721 F_3 plants selected from among the 433 F_2 families grown there, were labeled for date of heading. At least one plant was labeled in each family and for the awnless, apically-awnletted and awned strains several plants were labeled in the most promising strains. The plant labeled usually was the earliest to head. Very few strains appeared as uniform in date of heading as the parents. The data thus obtained in F_3 in comparison with the parents are shown in Table XV and graphically in Figure 2.

The average date of heading for the F_3 selections was June 25, that of Hard Federation June 24, and that of Kota July 2. As earliness was one of the principal characters for which selections were made it is apparent that the desired earliness is being obtained.

TABLE XV.—*Date of heading of 721 F_3 plants of the Kota-Hard Federation cross, in comparison with the parents, grown at Mandan, N. Dak., in 1923*

Frequency classes	F_3 hybrid		Hard Federation		Kota	
	Number	Percentage	Number	Percentage	Number	Percentage
June 20	58	8.0	11	11.6		
23	265	36.8	33	34.7		
26	324	44.9	47	49.5		
29	41	5.7	4	4.2	26	34.7
July 2	25	3.5			42	56.0
5	6	0.8			7	9.3
8	2	0.3				
	721	100.0	95	100.0	75	100.0

HEIGHT OF PLANT

Another growth character, height of plant, is an important economic factor in wheat production, because it may determine the method or ease of harvesting. It is inherited in the same manner as other characters.

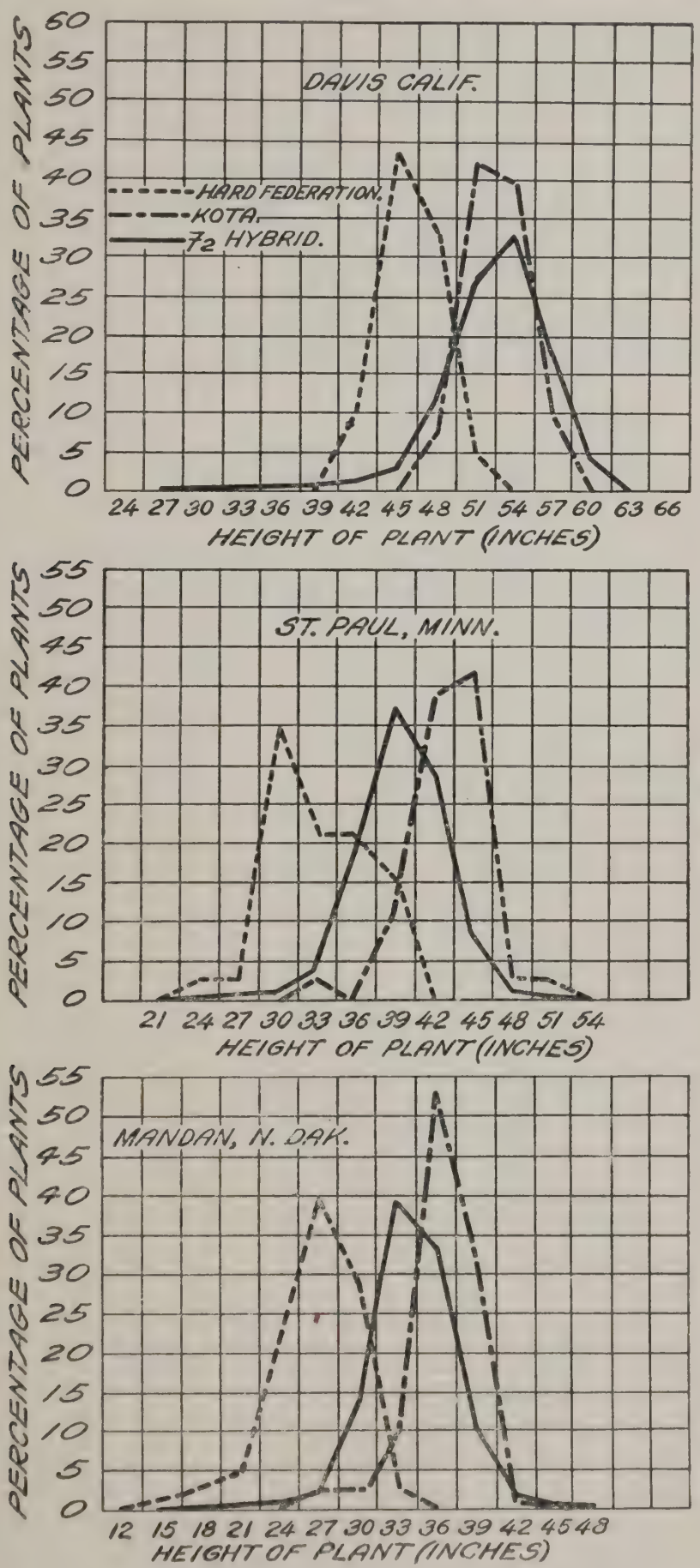
DAVIS CALIF.
HARD FEDERATION.
KOTA.
F_2 HYBRID.
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55 60
24 27 30 33 36 39 42 45 48 51 54 57 60 63 66
HEIGHT OF PLANT (INCHES)
ST. PAUL, MINN.
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55
21 24 27 30 33 36 39 42 45 48 51 54
HEIGHT OF PLANT (INCHES)
MANDAN, N. DAK.
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55
12 15 18 21 24 27 30 33 36 39 42 45 48
HEIGHT OF PLANT (INCHES)

FIG. 3.—Frequency distribution, by height of plant, of F_2 hybrids and of the Kota and Hard Federation parents at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922

Freeman (11), in a durum-common wheat cross, found the F_2 hybrids taller than the tallest parent and a wide segregation for height in the F_2. There was greater variability in the shorter progenies, indicating a complex character.

In the present study, the height of the individual F_2 plants was measured at harvest time at the three stations where the material was grown. Plate 2 shows the writer taking height notes at Davis, Calif. Height was measured from the base of the culms to the tip of the spike, not including the awns of awned strains. The frequency distribution by height classes, comparing F_2 plants with the parents, is shown in Table XVI and graphically in Figure 3. Only at Davis, Calif., were reciprocal crosses studied for this character.

TABLE XVI.—*Segregation of 2,532 F_2 plants of the Kota-Hard Federation reciprocal crosses, in comparison with parents, into 3-inch classes for height of plant, when grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and height classes (inches)	Number and percentage of plants on height of plant							
	F_2 hybrids				Hard Federation		Kota	
	Kota × Hard Federation	Hard Federation × Kota	Total	Percentage	Number	Percentage	Number	Percentage
DAVIS, CALIF.								
30		1	1	0.1				
33		2	2	.2	1	2.6		
36	1	3	4	.4	1	2.6		
39	4	3	7	.8	1	2.6		
42	4	7	11	1.2	4	10.2		
45	14	22	36	4.0	17	43.6		
48	49	70	119	13.3	13	33.3	3	7.5
51	85	145	230	25.6	2	5.1	17	42.5
54	100	197	297	33.1			16	40.0
57	54	99	153	17.0			4	10.0
60	12	24	36	4.0				
63	1	1	2	0.2				
Total	324	574	898	99.9	39	100.0	40	100.0
ST. PAUL, MINN.								
24	1			.2	1	3.1		
27	3			.9	1	3.1		
30	8			1.4	11	34.4		
33	22			3.9	7	21.9	1	2.8
36	101			17.8	7	21.9		
39	211			37.3	5	15.6	4	11.1
42	163			28.8			14	38.9
45	46			8.1			13	36.1
48	7			1.2			1	2.8
51	2			.4			1	2.8
Total	566			100.0	32	100.0	36	100.0
MANDAN, N. DAK.								
15					1	1.5		
18	1			.1	2	2.9		
21	3			.3	3	4.4		
24	6			.5	14	20.6		
27	24			2.2	27	39.7	2	2.4
30	145			13.6	19	27.9	2	2.4
33	418			39.2	2	2.9	7	8.5
36	351			32.8			5	6.1
39	103			9.7			27	32.9
42	21			2.0			6	7.3
45	1			.1			1	1.2
Total	1,068			100.0	68	100.0	82	99.8

TABLE XVII.—*Mean height, standard deviation, and coefficient of variation for height of plant of F_2 hybrids and for the Kota and Hard Federation varieties, grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922*

Station and material	Mean height (inches)	Standard deviation	Coefficient of variation
DAVIS, CALIF.			
F_2 hybrid	52.43±0.09	3.806±0.061	7.259±0.116
Kota	52.58± .25	2.323± .175	4.418± .333
Hard Federation	45.31± .38	3.517± .269	7.762± .593
ST. PAUL, MINN.			
F_2 hybrid	39.48± .10	3.553± .071	8.999± .180
Kota	43.08± .34	3.013± .240	8.994± .556
Hard Federation	32.72± .48	4.064± .343	12.421±1.047
MANDAN, N. DAK.			
F_2 hybrid	34.11± .05	3.144± .065	9.217± .190
Kota	36.40± .19	2.599± .137	7.140± .376
Hard Federation	26.69± .28	3.379± .195	12.660± .732

The frequency distribution shown in Table XVII at Davis, Calif., indicates no maternal influence on height of plant in the reciprocal crosses. The mean heights and their difference, together with their probable errors, are as follows:

Kota × Hard Federation	52.28±0.15
Hard Federation × Kota	52.52± .12
Difference	.24± .19

The difference of 0.24 ± 0.19 of an inch is not only small but not significant as it is only 1.26 times its probable error, indicating odds of about 1.5 : 1.

Evidence that tall plants are dominant is shown by all F_2 data. At the three points the hybrids tend to the tallness of the Kota parent. The variability of the hybrids in comparison with the parents is of interest. Table XVII shows the F_2 progeny to exceed Kota in variability at all three points but to be exceeded in variability by the short Hard Federation parent. The disparity of numbers for the parent reduces the significance of differences in variability. The height is shown to decrease in relation to increasing unfavorable conditions, such as rust and drought.

Under conditions of both rust and drought, F_3 material was studied further in 1923 at Mandan, N. Dak. In all, 726 hybrid plants were measured for height, among 433 F_2 families grown. At least one plant was measured from each family and in the most promising rows several plants were measured. These were the same as those labeled for date of heading, the object being to obtain both early and tall plants. The data obtained at Mandan in F_3 are given in Table XVIII and graphically in Figure 4.

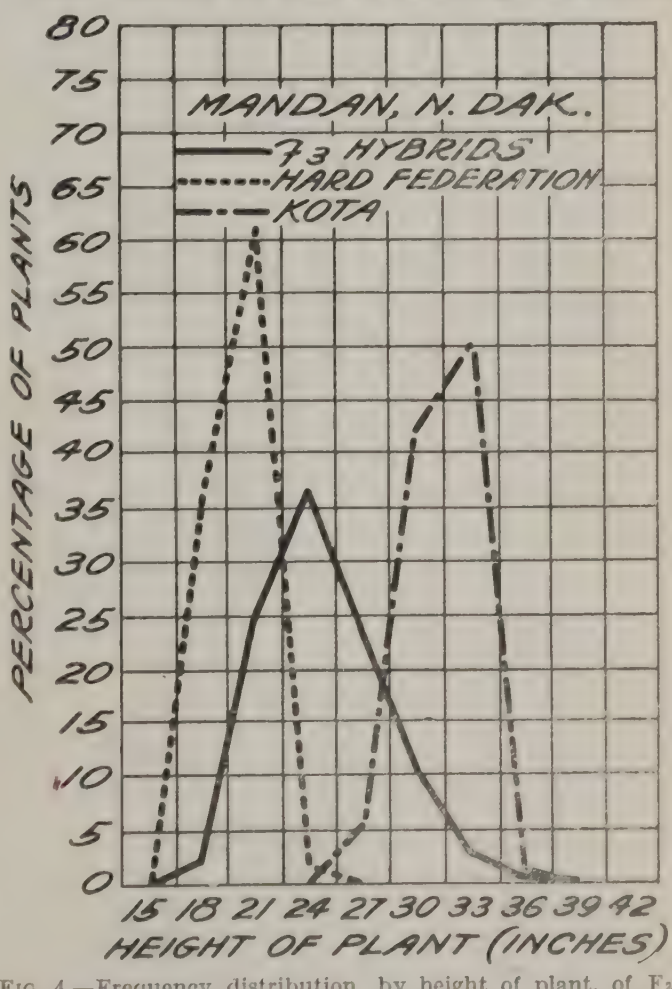

FIG. 4.—Frequency distribution, by height of plant, of F_2 selections and of the Kota and Hard Federation parents at Mandan, N. Dak., in 1923

TABLE XVIII.—*Height of 726 F_3 plants of the Kota-Hard Federation cross, in comparison with the parents, when grown at Mandan, N. Dak., in 1923*

Frequency distribution by height classes (inches)	F_3 hybrid		Hard Federation		Kota	
	Number	Percentage	Number	Percentage	Number	Percentage
18	18	2.5	35	36.8		
21	174	24.0	58	61.1		
24	268	36.9	2	2.1		
27	170	23.4			4	5.3
30	74	10.2			32	42.7
33	20	2.8			38	50.7
36	2	.3			1	1.3
Total	726	100.1	95	100.0	75	100.0

A severe June drought occurred at Mandan, causing a shortening of both hybrids and parents. Rains early in July enabled late varieties or late-sown wheat to reach a more normal height. This droughty condition affected the F_3 results. It will be noted that the majority of F_3 hybrids tend to the shortness of the Hard Federation parent, which is the reverse of the F_2 results. It is concluded that tallness may be partially dominant but due principally to heterosis and easily influenced by different environmental conditions. As most of the selections were as early as Hard Federation, the desired increase in height is being obtained in some cases even under conditions of severe drought. The average height of the F_3 selections was 25 inches, that of Hard Federation 20 inches, and that of Kota 31 inches. The hybrids were more than twice as variable as the parents, the coefficient of variability for the hybrids and parents being as follows:

F_3 hybrids	13.365 ± 0.237
Hard Federation	$6.823 \pm .334$
Kota	$5.624 \pm .310$

STEM-RUST INFECTION

Resistance to stem rust, *Puccinia graminis tritici*, is the most economically important physiological character studied in this cross, as severe losses from stem-rust infection are common in the northern spring-wheat section.

Several investigators, both plant breeders and plant pathologists, are studying resistance to this disease. Hayes and Stakman (*21*) have reviewed the investigations of these workers. Stakman and Levine (*33*) have shown that there are 37 physiologic forms of stem rust of wheat which are identified by their parasitic action on 12 differential hosts. This is thought to account for much of the disagreement in field results. Aamodt (*2*), in a study of Kanred × Marquis, found Kanred immune to several of these specialized forms and susceptible to others. In greenhouse studies with the immune strains, immunity was found to be dominant. There were no intermediates. The experiment indicated that the reaction to the several forms was inherited as a unit. Hayes and Aamodt (*20*) have shown under field conditions that in the Marquis-Kota cross the resistance of Kota was recessive. No definite genetic ratio was determined, resistant F_3 families occurring in the proportion of about 1 to 7.73. The resistant strains were as resistant as Kota.

In the Kota-Hard Federation cross, the present study in the field also shows susceptibility to be dominant and resistance recessive. At St. Paul the plants were inoculated with nine different specialized forms of stem rust common to the northern spring-wheat region. At Mandan, N. Dak., natural infection occurred and it is not known how many forms of rust were present there. No rust occurred at Davis, Calif. Abundant infections were obtained at both St. Paul and Mandan, but the injury was not great at either place. Infection at Mandan was a little more abundant than at St. Paul. Rust notes were taken on individual plants at both points. The data are given in Table XIX and graphically shown in Figure 5. Extreme degrees of infection obtained on F_2 plants grown at Mandan are shown in Plate 3. The infection on the resistant plant was recorded as 2 per cent and that on the susceptible plant as 95 per cent. Degrees of infection intermediate between these two extremes were obtained on the hybrid material at both points.

Extreme degrees of stem-rust infection of F_2 hybrids of the Kota-Hard Federation cross at Mandan, N. Dak., in 1922; (A) 2 per cent of infection and (B) 95 per cent of infection

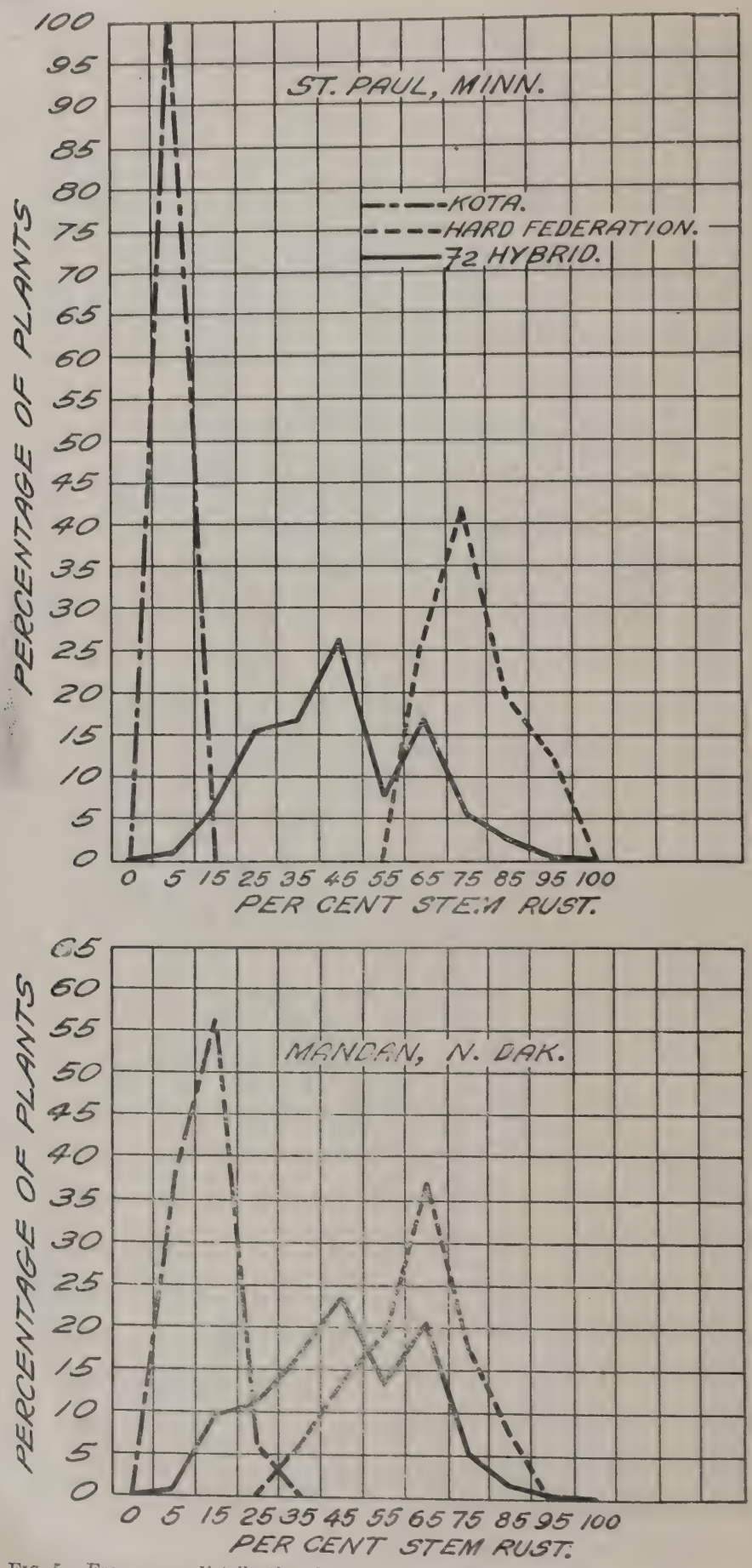
ST. PAUL, MINN.
KOTA.
HARD FEDERATION.
72 HYBRID.
PERCENTAGE OF PLANTS
0 5 15 25 35 45 55 65 75 85 95 100
PER CENT STEM RUST.
MANDAN, N. DAK.
PERCENTAGE OF PLANTS
0 5 15 25 35 45 55 65 75 85 95 100
PER CENT STEM RUST.

FIG. 5.—Frequency distribution, by percentage of stem-rust infection, of F_2 hybrids and of the Kota and Hard Federation parents at St. Paul, Minn., and Mandan, N. Dak., in 1922

TABLE XIX.—*Segregation of 1722 F_2 plants of the Kota-Hard Federation cross, in comparison with parents, into 10 per cent infection classes for stem-rust infection, when grown at St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and rust infection classes (per cent)	Number and percentage of plants on stem-rust infection					
	F_2 hybrids		Hard Federation		Kota	
	Number	Percentage	Number	Percentage	Number	Percentage
ST. PAUL, MINN.						
5	9	1.6			36	100.0
15	40	7.1				
25	85	15.1				
35	92	16.3				
45	150	26.6				
55	43	7.6				
65	96	17.0	8	25.8		
75	31	5.5	13	42.0		
85	16	2.8	6	19.4		
95	2	.4	4	12.9		
Total	564	100.0	31	100.1	36	100.0
MANDAN, N. DAK.						
5	7	.6			31	37.8
15	109	9.4			46	56.1
25	115	9.9			5	6.1
35	173	14.9	4	5.9		
45	274	23.7	9	13.2		
55	160	13.8	13	19.1		
65	235	20.3	25	36.8		
75	63	5.4	12	17.6		
85	20	1.7	5	7.5		
95	2	.2				
Total	1,158	99.9	68	100.1	82	100.0

TABLE XX.—*Segregation of 6,052 F_2 plants of Kota-Hard Federation and reciprocal crosses into resistant and susceptible classes for stem-rust infection, when grown at St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and cross	Number of F_1 families	Number and percentage of F_2 plants				Total number	Deviation from 15:1	Probable error
		Susceptible		Resistant				
		Number	Percentage	Number	Percentage			
ST. PAUL, MINN.								
Kota×Hard Federation	30	864	92.0	75	8.0	939	16	5.00
Hard Federation×Kota	40	963	93.3	69	6.7	1,032	4	5.24
Total	70	1,827	92.7	144	7.3	1,971	21	7.24
MANDAN, N. DAK.								
Kota×Hard Federation	28	1,946	93.2	143	6.8	2,089	12	7.48
Hard Federation×Kota	38	1,876	94.2	116	5.8	1,992	9	7.28
Total	66	3,822	93.7	259	6.3	4,081	4	10.46
Grand total	75	5,649	93.3	403	6.7	6,052	25	12.70

The rust notes taken on individual F_2 plants shown in Table XIX were on the same plants labeled for date of heading and measured for height. These were of the Kota × Hard Federation cross. Kota showed resistance, with an average of only 3.5 per cent infection at St. Paul and 12 per cent at Mandan. Hard Federation was susceptible, with an average infection of

77 per cent at St. Paul and 68 per cent at Mandan. The F_2 hybrids showed from 5 to 95 per cent of infection at both points, averaging 45 per cent at St. Paul and 46 per cent at Mandan. There was no distinct separation between resistant and susceptible strains.

Additional material was studied for resistance, upon which individual rust notes were recorded only on plants about as resistant as the Kota parent or having an estimated infection of 15 per cent or less. This was thought to be about the maximum amount of infection which could occur without damage. By taking this estimated infection of 15 per cent or less as resistant and grouping the remainder as susceptible at both St. Paul and Mandan, F_2 results were found to agree very closely to a 2-factor hypothesis. The data obtained are shown in Table XX.

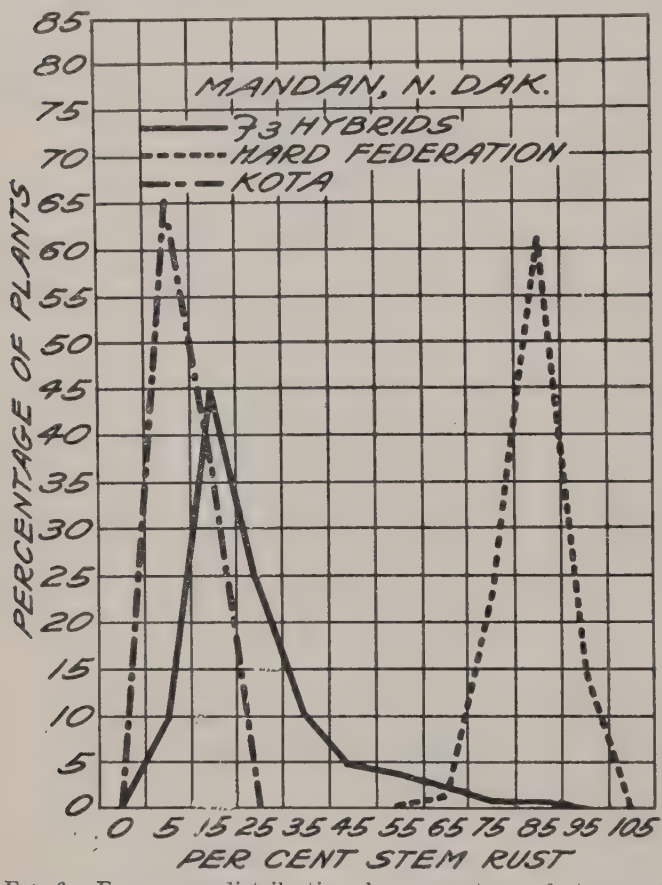

FIG. 6.—Frequency distribution, by percentage of stem-rust infection, of F_3 selections and of the Kota and Hard Federation parents at Mandan, N. Dak., in 1923

Table XX shows that, on the arbitrary division made, there is slight evidence of maternal influence. Where the susceptible Hard Federation was used as the female parent, a close fit to the 15:1 ratio was obtained in both cases. Where the resistant Kota was used as the female parent a greater proportion of resistant plants was obtained and at St. Paul the deviation from the expected ratio was greater than three times the probable error. The grand average for both crosses at both stations, however, shows that a very close fit was obtained in F_2 for the assumption that two Mendelian factors are involved for stem-rust resistance in this cross.

At Mandan, N. Dak., in 1923, the 724 F_3 plants, which were labeled for date of heading and measured for height, were examined individually and the infection of stem rust estimated. The data obtained are given in Table XXI and shown graphically in Figure 6.

TABLE XXI.—*Stem-rust infection on 724 F_3 plants of the Kota-Hard Federation and reciprocal crosses, and comparison with the parents, grown at Mandan, N. Dak., in 1923*

Frequency classes in percentage of stem-rust	F_3 hybrid		Hard Federation		Kota	
	Number	Percentage	Number	Percentage	Number	Percentage
5	68	9.4			49	65.3
15	326	45.0			26	34.7
25	175	24.2				
35	71	9.8				
45	33	4.6				
55	27	3.7				
65	16	2.2	1	1.1		
75	5	0.7	22	23.2		
85	3	0.4	58	61.1		
95			14	14.7		
Total	724	100.0	95	100.1	75	100.0

The average infection of these F_3 hybrids was 23 per cent, compared with 7 per cent for Kota and 84 per cent for Hard Federation. The average infection of Marquis grown in check rows in the same experiment was 49 per cent. Considerable resistance has been obtained in the selected plants, although the average percentage is not as great as that of the Kota parent. However, it is much greater than that of Marquis.

Unfortunately the resistant plants which were separated so that they appeared to segregate in F_2 in a 1:15 ratio were not found to breed true for resistance in the F_3. A large number of the so-called resistant F_2 plants were grown at both St. Paul and Mandan in F_3, and not one could be said to be homozygous for resistance. All of the F_3 material grown at St. Paul and Mandan was examined for resistant plants, and the percentage of these to the total by F_2 frequency classes has been determined. Only resistant strains were grown at St. Paul, while at Mandan both resistant and susceptible strains were grown. The resistant group of F_2 plants noted for stem-rust infection has been classified into 3 per cent classes and the susceptible group into 10 per cent classes. The data on segregation in the F_3 according to these groups and classes are given in Table XXII.

The data for the resistant groups show that there was a much larger proportion of resistant plants at Mandan than at St. Paul. This can be attributed to the earlier and more severe rust epidemic which occurred at St. Paul. At Fargo, N. Dak., where a few of the resistant F_2 selections were grown and where rust was much more severe than at St. Paul, there did not appear to be any F_3 plants which could be classed as resistant or as having the resistance of Kota. No definite data were taken on the material at Fargo. At Mandan, where only fairly severe infection occurred, definite data were taken on F_3 of both the resistant and the susceptible F_2 groups. At St. Paul only the resistant group was grown. When it is divided into five 3 per cent classes, as shown in Table XXII, there is seen to be a general, but not a definite, decrease in the percentage of resistant F_3 plants. The decrease is from 18.8 per cent for the 2 per cent class to 5 per cent for the 14 per cent class. At Mandan there was no general decrease among the five classes of the resistant group. The resistant

TABLE XXII.—*Segregation of 10,042 F_3 plants of the Kota-Hard Federation and reciprocal crosses grown at St. Paul, Minn., and Mandan, N. Dak., in 1923, arranged on the basis of infection of the F_2 resistant and susceptible groups in 1922*

Source, group, and infection class in F_2 (per cent)	Number of F_2 families	Number of F_3 plants			Per cent resistant
		Susceptible	Resistant	Total	
ST. PAUL, MINN.[a]					
Resistant:					
(1–3) 2	2	26	6	32	18.8
(4–6) 5	17	213	38	251	15.1
(7–9) 8	20	291	15	306	4.9
(10–12) 11	48	665	72	737	9.8
(13–15) 14	49	705	37	742	5.0
Total	136	1,900	168	2,068	8.1
MANDAN, N. DAK.					
Resistant:					
(1–3) 2	10	158	84	242	34.7
(4–6) 5	40	773	191	964	19.8
(7–9) 8	27	528	99	627	15.8
(10–12) 11	116	1,987	725	2,712	26.7
(13–15) 14	80	1,449	393	1,842	21.3
Total	273	4,895	1,492	6,387	23.4
Susceptible:					
(16–20) 18	20	449	29	478	6.1
(21–30) 25	31	728	14	742	1.9
(31–40) 35	39	896	22	918	2.4
(41–50) 45	20	474	3	477	.7
(51–60) 55	20	517	3	520	.6
(61–70) 65	14	331	4	335	1.2
(71–80) 75	6	150	0	150	0
(81–90) 85	2	35	0	35	0
Total	152	3,580	75	3,655	2.1
Mandan total	425	8,475	1,567	10,042	15.6

[a] F_3 data at St. Paul were obtained by Olaf S. Aamodt.

group averaged 23.4 per cent of resistant plants at Mandan and only 8.1 per cent at St. Paul.

The susceptible group at Mandan showed that F_2 plants having from 16 to 70 per cent infection produced a few resistant plants. All F_2 plants grown which had 71 per cent or more of infection, did not produce any resistant plants. The susceptible class produced as a whole only 2.1 per cent of resistant plants. Among the entire 10,042 F_3 plants grown at Mandan, 1,567, or 15.6 per cent, were classified as resistant.

Of the 75 F_4 selections grown at Mandan in 1923, 31 were estimated to have an average rust infection of 15 per cent or less. These were all from what appeared to be resistant F_3 plants at St. Paul or Mandan in 1922. Of the 31 only 10 appeared as resistant as Kota (average of 5 per cent), including 4 which were noted to be more resistant. There is evidence, therefore, that F_4 strains can be obtained which are homozygous for stem-rust resistance. Whether they will be as resistant or more resistant than Kota under conditions of very severe rust remains to be determined.

YIELD OF PLANTS

Yield may be considered as a character complex affected by environment and by most of the morphological and physiological characters of the plant. Engledow and Wadham (*10*) state that—

> Cereal yield is controlled by a great number of factors which are themselves complex and imperfectly understood. In approaching the yield problem it is convenient to arrange these in broad categories which may thus be designated: (1) Soil, (2) climate, (3) agricultural practice, (4) disease and damage, (5) botanical variety or form.

Beaven (*3*) concludes that—

> The problem of the cereal breeder is to discover the relation between the different structures of the individual plant and the probable quantity of saleable produce per acre. The predominant factor of productivity in cereals is the seed-forming energy of the individual plants composing the crop.

Yields of F_2 and F_3 plants were obtained in the present study. The plants were definitely spaced, harvested individually, threshed, and the grain weighed to tenths of grams. In the F_2 yields were obtained at St. Paul and Mandan. The data obtained are given in Table XXIII and shown graphically in Figure 7.

TABLE XXIII.—*Segregation of 1,699 F_2 plants of the Kota-Hard Federation crosses, in comparison with their parents, into 1-gram classes for yield of plant, at St. Paul, Minn., and Mandan, N. Dak., in 1922*

Source and yield classes (grams)	Number and percentage of plants					
	F_2 hybrid		Hard Federation		Kota	
	Number	Percentage	Number	Percentage	Number	Percentage
ST. PAUL, MINN.						
0.5	28	5.2	5	31.2		
1.5	83	15.3	7	43.7		
2.5	91	16.8	2	12.5	1	5.6
3.5	97	17.9	2	12.5	1	5.6
4.5	82	15.1			2	11.1
5.5	67	12.4			2	11.1
6.5	44	8.1			4	22.2
7.5	24	4.4			2	11.1
8.5	13	2.4			3	16.7
9.5	7	1.3			2	11.1
10.5	3	.6			1	5.6
13.5	1	.2				
15.5	1	.2				
Total	541	99.9	16	99.9	18	100.1
MANDAN, N. DAK.						
0.5	16	1.4	10	23.8	1	3.3
1.5	83	7.2	14	33.3	2	6.7
2.5	196	16.9	11	26.2	10	33.3
3.5	278	24.0	5	11.9	8	26.7
4.5	264	22.8	2	4.8	6	20.0
5.5	166	14.3			2	6.7
6.5	93	8.0			1	3.3
7.5	35	3.0				
8.5	17	1.5				
9.5	5	.4				
10.5	3	.3				
11.5	1	.1				
12.5	1	.1				
Total	1,158	100.0	42	100.0	30	100.0

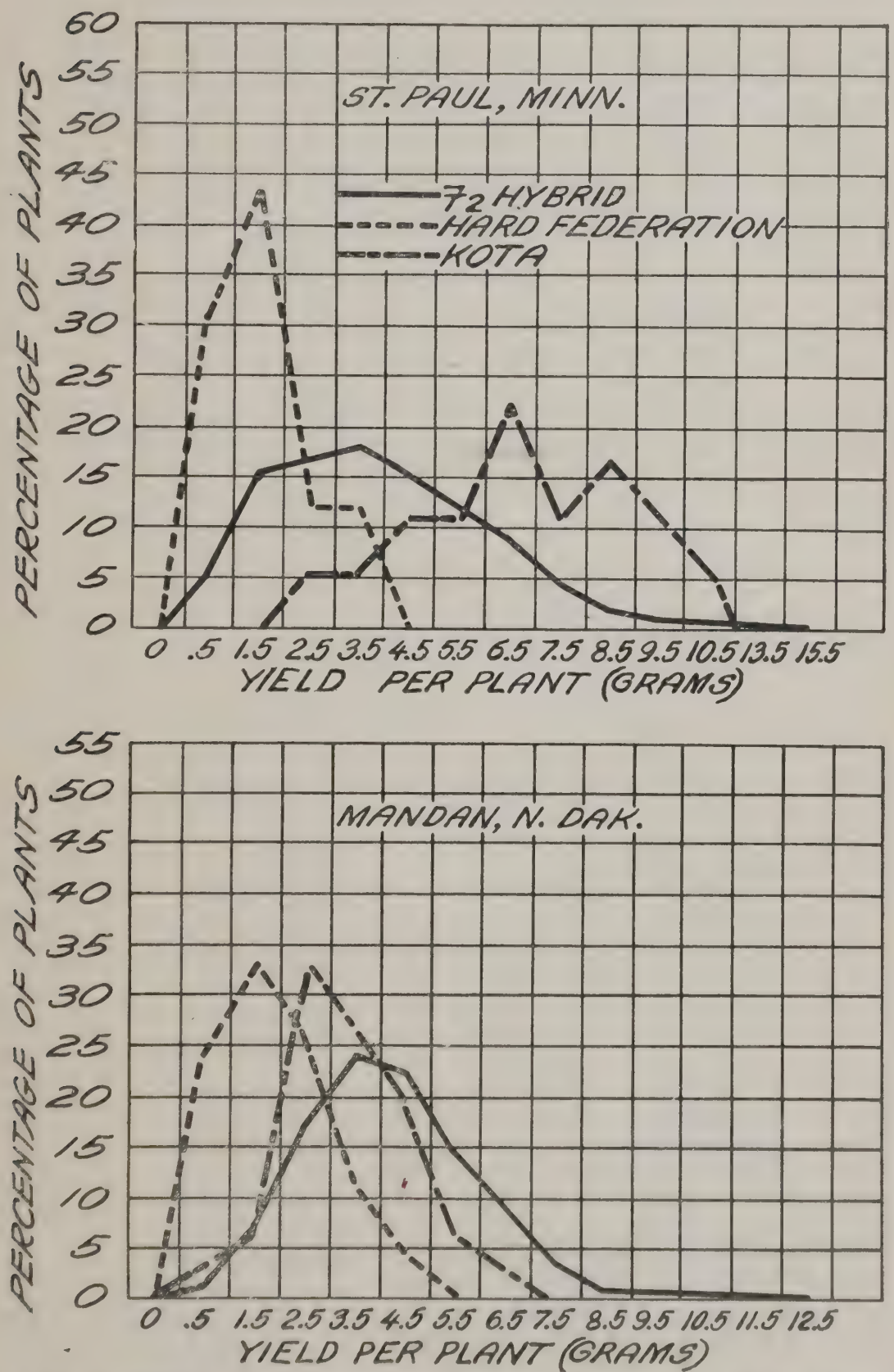
ST. PAUL, MINN.
F_2 HYBRID
HARD FEDERATION
KOTA
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55 60
0 .5 1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5 9.5 10.5 13.5 15.5
YIELD PER PLANT (GRAMS)
MANDAN, N. DAK.
PERCENTAGE OF PLANTS
0 5 10 15 20 25 30 35 40 45 50 55
0 .5 1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5 9.5 10.5 11.5 12.5
YIELD PER PLANT (GRAMS)

FIG. 7.—Frequency distribution, by yield of plant, of F_2 hybrids and of the Kota and Hard Federation parents at St. Paul, Minn., and Mandan, N. Dak., in 1922

The data show a wide range in variation in the yield of F_2 plants. The number of parent plants threshed for comparison is small, especially at St. Paul. The average yield, standard deviation, and coefficient of variation of F_2 hybrids and parents are shown in Table XXIV. It appears important that at Mandan the average yield of the hybrids exceeds that of both parents and that at both points the coefficient of variation is greater for the hybrids than for Kota, the high-yielding parent. Because of the disparity of numbers of the parents and of the low mean yield of Hard Federation the standard deviation apparently is the best measure of variability. It shows at both points that the hybrids are considerably more variable than either parent.

At Mandan, N. Dak., in 1923, the threshed grain from 900 F_3 plant selections was weighed and the yields are compared with those of the parents in Table XXV and shown graphically in Figure 8.

TABLE XXIV.—*Mean yield, standard deviation, and coefficient of variation for yield of plants of F_2 hybrids, and of the parents Kota and Hard Federation varieties, grown at St. Paul, Minn., and Mandan, N. Dak., in 1922*

Station and material	Mean yield	Standard deviation	Coefficient of variation
ST. PAUL, MINN.	*Grams*		
F_2 hybrid	3.97±0.06	2.215±0.045	55.793±1.144
Kota	6.78± .34	2.129± .239	31.401±3.530
Hard Federation	1.56± .16	.966± .115	61.923±7.383
MANDAN, N. DAK.			
F_2 hybrid	4.15± .03	1.713± 0.24	41.277± .579
Kota	3.37± .16	1.284± .112	38.101±3.318
Hard Federation	1.90± .12	1.114± .082	58.632±4.315

The data show that in segregation for yield the F_3 selections made in 1923 were less variable, in comparison with the parent than were the F_2 selections. The mean yield, standard deviation, and coefficient of variation for the F_3 hybrids and the parents are shown in Table XXVI.

Table XXVI shows that the mean yield of the hybrids is slightly but not significantly less than that of Kota, the higher yielding parent, the difference being 0.19±0.10 grams. The standard deviation and coefficient of variation also are less than for Kota. The mean yield of a number of Marquis plants grown as checks was 3.03 grams, which was slightly but not significantly less than the hybrid selections.

As practically all the F_3 plant rows were heterozygous for one or another of the plant characters studied, none of the rows was harvested for yield. As the mean yield of each selection was not obtained, it is not possible to show the segregation for yield in F_3 according to F_2 classes.

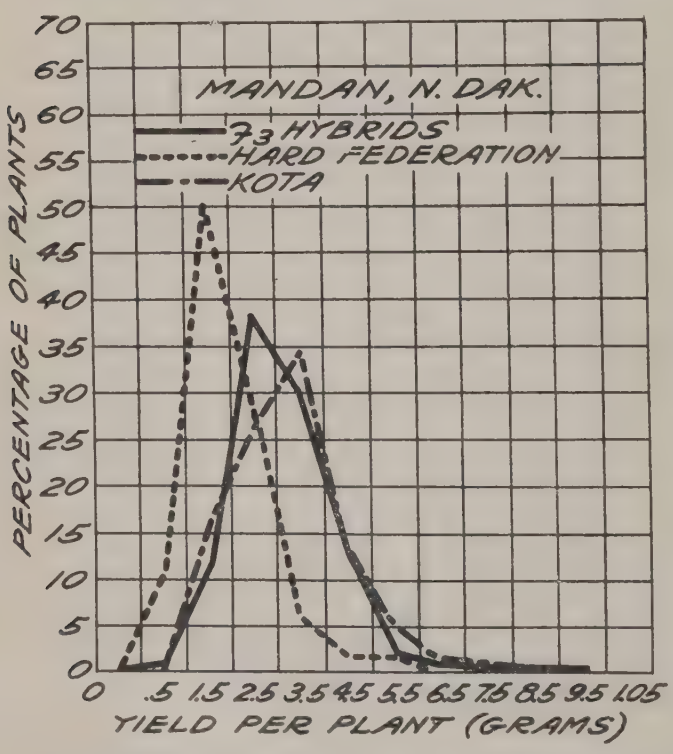

FIG. 8.—Frequency distribution, by yield of plant, of F_3 selections and of the Kota and Hard Federation parents at Mandan, N. Dak., in 1923

TABLE XXV.—*Yield of 900 F_3 plants of the Kota-Hard Federation and reciprocal crosses, in comparison with that of the parents, grown at Mandan, N. Dak., in 1923*

Frequency classes yield (grams)	F_3 hybrids		Hard Federation		Kota	
	Number	Percentage	Number	Percentage	Number	Percentage
0.5	14	1.6	11	11.6		
1.5	114	12.7	48	50.5	12	16.4
2.5	346	38.4	26	27.4	20	27.4
3.5	274	30.4	6	6.3	25	34.2
4.5	115	12.8	2	2.1	10	13.7
5.5	22	2.4	2	2.1	4	5.5
6.5	10	1.1			1	1.4
7.5	4	0.4			1	1.4
8.5	1	0.1				
Total	900	99.9	95	100.0	73	100.0

TABLE XXVI.—*Mean yield, standard deviation, and coefficient of variation for yield of plants of F_3 hybrids and for the Kota and Hard Federation parent varieties, grown at Mandan, N. Dak. in 1923*

Station and material	Mean yield	Standard deviation	Coefficient of variation
	Grams		
F_3 hybrids	3.05±0.03	1.119±0.018	36.689±0.583
Kota	3.24± .10	1.250± .070	38.580±2.154
Hard Federation	1.93± .07	.991± .048	51.347±2.512

SEGREGATION IN THE F_4 FOR FACTORS OF QUALITY

As the quality of a new hard red spring wheat is of importance equal to that of yield, it is very desirable to determine at the earliest practical time the segregation of hybrid selections for important quality-affecting factors. In the present investigations an effort is being made to study certain quality factors, starting with grain from F_4 selections of the hybrids. Lack of funds for this work limits the number of determinations, so the results here given can be considered only as preliminary.

From the 75 F_4 selections grown at Mandan in 1923, grain from 30 of the most promising was selected for making tests for four quality factors. These are (1) viscosity, for the quality of the gluten; (2) crude protein of wheat and flour, for the quantity of gluten; (3) gasoline color reaction, for the color of the flour; and (4) ash, for quantity of ash in the flour. The quality and quantity of the gluten in hard red spring wheat is of great importance to the trade. Their objection to the yellowish color and high ash content of Kota also makes it desirable to give careful consideration to segregation for these factors in hybrids having Kota as a parent.

VISCOSITY

Viscosity determinations have been suggested recently by several workers as a means of approximately evaluating the relative quality of the gluten in flours, without baking. Principal among the studies in this field of colloidal chemistry are those by Ostwald (*25*), and Luers and Ostwald (*23*) of Germany, and by Weaver and Goldtrap (*37*), Gortner and Sharp (*16*), and Sharp and Gortner (*29*) in the United States. A standard method has not yet been evolved.

The writer conducted some preliminary trials (unpublished) under the direction of Dr. C. H. Bailey at the University of Minnesota, using the improved MacMichael viscosimeter. Twenty grams of flour of each sample were mixed with 100 cc. of distilled water and poured into 900 cc. of water. The flour particles were kept in suspension for 50 minutes by intermittent agitation. After settling for 10 minutes the extract was decanted and 500 cc. of water added to the residue and agitated. The suspension again was allowed to settle and decanted to 100 cc. The washing removed a large part of the soluble electrolytes. The 100 cc. of residue was poured into the bowl of the MacMichael viscosimeter.

A 2-centimeter cylindrical bob was used, suspended by a No. 30 wire. The viscosity was determined at once and after the addition of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 cc. of normal lactic acid.

One hundred and twelve samples were studied, including 50 varieties of wheat representing the five commercial classes, Hard Red Spring, Durum, Hard Red Winter, Soft Red Winter, and White, obtained from 11 agricultural experiments stations in 10 Western States. The wheat samples had been milled and baked previously in the Milling Investigations Laboratory of the Grain Division of the Bureau of Agricultural Economics, United States Department of Agriculture, at Washington, D. C.

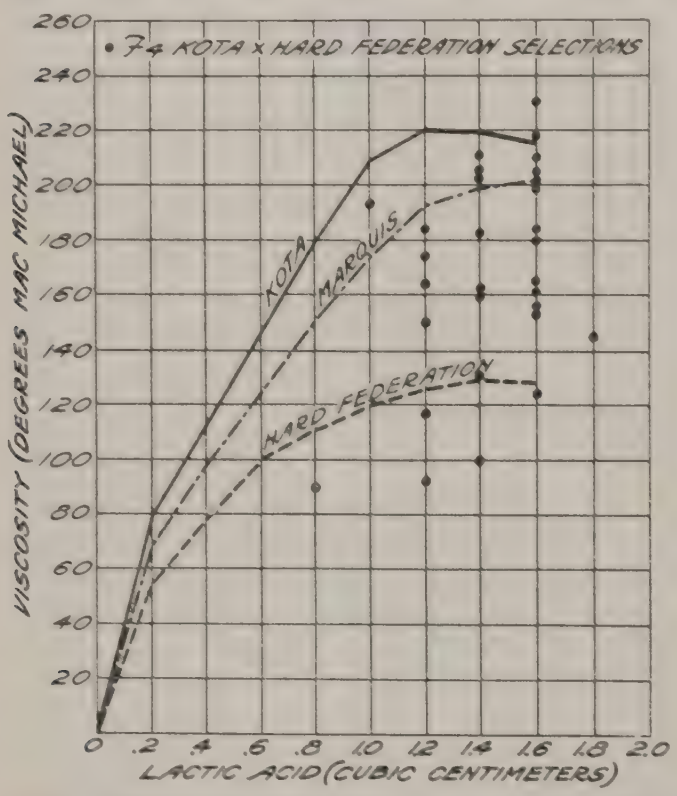

FIG. 9.—Viscosity curves for the Kota, Marquis, and Hard Federation varieties, and maximum viscosity for 30 F_4 Kota × Hard Federation selections grown at Mandan, N. Dak., in 1923

The viscosity as determined by constant readings, in degrees MacMichael, was correlated with loaf volume, in cubic centimeters, and the important and significant positive correlation 0.368 ± 0.055 obtained. The viscosity of the same samples correlated with crude-protein content of the wheat ($N \times 5.7$, 13.5 per cent moisture) gave a less important correlation of 0.287 ± 0.058. The correlation between crude protein and loaf volume for the same 112 samples was negative, -0.066 ± 0.063 and not important or significant. These experiments, therefore, confirmed the conclusions of previous studies that the viscosity determinations indicated strength of gluten, although there was also a fairly good correlation between viscosity and crude-protein content.

This preliminary study led to a trial of the viscosity test for determining the quality of promising F_4 selections in the present hybrid study. The limited amount of grain produced from a plant selection prevents the making of a baking test. Seventy-five grams of wheat of each of 30 F_4 selections were milled as uniformly as possible and produced approximately 35 grams of straight flour. Because of the small amount of grain, uniform milling was very difficult and the results were not entirely satisfactory. The flour obtained was tested for viscosity, crude protein, gasoline color, and ash. The viscosity trials were conducted by the writer by the method decribed above. Flour from the Kota and Hard Federation parent varieties, as well as Marquis, grown in check rows, were tested in duplicate in a similar manner. The viscosities obtained with these varieties, with increasing amounts of lactic acid until maximum viscosity was reached, are shown in Figure 9. The distribution of the maximum viscosity of each of the 30 hybrid strains is indicated by dots in the Figure. The segregation by 20-degree frequency classes also is shown in Table XXVII.

The data show a wide segregation with most of the hybrids intermediate between the parents, although one exceeded Kota and five were lower than Hard Federation. The average maximum viscosity for the 30 hybrids was 170 degrees MacMichael, compared with averages of 220, 203, and 129 degrees MacMichael, respectively, for duplicate samples of Kota, Marquis, and Hard Federation. The disparity in numbers prevents any conclusion regarding the mode of inheritance of strength of flour, but there is distinct evidence of segregation and the viscosity test furnishes a promising method of attack by which to breed wheat for gluten quality.

TABLE XXVII.—*Segregation of 30 F_4 selections of the Kota-Hard Federation and reciprocal crosses, together with the parents and Marquis, for viscosity in degrees MacMichael, at Mandan, N. Dak., in 1923*

Class	F_4 hybrids		Hard Federation	Kota	Marquis
	Number	Percentage	Number	Number	Number
100	3	10.0			
120	2	6.7	1		
140	2	6.7	1		
160	8	26.7			
180	5	16.7			
200	6	20.0			2
220	3	10.0		2	
240	1	3.3			
Total	30	100.1	2	2	2

CRUDE-PROTEIN CONTENT

The quantity of crude protein in the wheat and flour is a standard index of the quantity of gluten. Determinations in this study were made by the method described by Shollenberger, Marshall, and Coleman (*31*) (N.×5.7, basis 13.5 per cent moisture) in the Research Laboratory of the Grain Division of the Bureau of Agricultural Economics. The results obtained on crude protein in the kernels are shown in Table XXVIII.

The data show evidence of segregation in crude-protein content of the kernels. The small numbers involved do not permit of any definite conclusion, but in general it appears that high crude-protein wheats can be obtained from this cross as most of the selections are equal or superior to Kota. Twenty-nine hybrid selections averaged 16.1 per cent crude protein in the wheat, while duplicate samples of Kota, Marquis, and Hard Federation averaged 15.9, 15.7, and 14.7 per cent, respectively.

TABLE XXVIII.—*Segregation of 29 F_4 selections of the Kota-Hard Federation and reciprocal crosses, together with the parents and Marquis, for crude protein of the kernels, at Mandan, N. Dak., in 1923*

Classes by crude protein content (per cent)	F_4 hybrids		Hard Federation	Kota	Marquis
	Number	Percentage	Number	Number	Number
14.5			1		
15.0	3	10.3	1		
15.5	6	20.7			1
16.0	11	37.9		2	1
16.5	4	13.8			
17.0	2	6.9			
17.5	3	10.3			
Total	29	99.9	2	2	2

The results obtained on crude protein in the flour are given in Table XXIX.

The data on crude protein in the flour also show evidence of segregation and, while the disparity of numbers prevents definite conclusions, it appears probable that in this cross hybrid selections can be obtained which exceed the parents in the quantity of their gluten. Twenty-eight hybrids averaged 15.6 per cent crude protein in the flour while duplicate samples of Kota, Hard Federation, and Marquis averaged 14.9, 14.6, and 14.3 per cent, respectively.

TABLE XXIX.—*Segregation of 28 F_4 selections of the Kota-Hard Federation and reciprocal crosses, together with the parents and Marquis, for crude protein of the flour, at Mandan, N. Dak., in 1923*

Classes by crude protein content (per cent)	F_4 hybrids		Hard Federation	Kota	Marquis
	Number	Percentage	Number	Number	Number
14.0	2	7.1	1	1	1
14.5	1	3.6			1
15.0	7	25.0	1		
15.5	10	35.7		1	
16.0	5	17.9			
16.5	1	3.6			
17.0	1	3.6			
17.5	0	.0			
18.0	1	3.6			
Total	28	100.1	2	2	2

FLOUR COLOR

The gasoline color test is considered the best practicable method of determining the color of flour. The method used in this study is described by Shollenberger, Marshall, and Coleman (*31*). A limited number of preliminary experiments have shown Kota to exceed Marquis in gasoline color value. No comparison had been made between Kota and Hard Federation. Because of the popular demand for white flour and bread there is a prejudice against flours with a creamy or yellowish color containing more carotin. As the trade finds this character objectionable in Kota it is important to determine if there is segregation for this character in Kota hybrids.

Gasoline color values have been determined on the flours of the 30 F_4 hybrids and on the duplicate samples of Kota, Hard Federation, and Marquis, previously discussed. The data are summarized by frequency classes in Table XXX.

The data show considerable segregation in the hybrids. The limited data do not show Kota to exceed Hard Federation or Marquis in color of flour and are not in accord with preliminary experiments and commercial findings. However, a considerable number and percentage of the hybrids have less color than the parents and Marquis, indicating segregation in the desired direction and the possibility of obtaining the desired results. The average gasoline color value for the 30 F_4 hybrids was 1.79 and that of Hard Federation, Kota, and Marquis 2.01, 1.74, and 1.94, respectively.

TABLE XXX.—*Segregation of 30 F_4 selections of the Kota-Hard Federation and reciprocal crosses, together with the parents and Marquis, for gasoline color value, at Mandan, N. Dak., in 1923*

Classes by gasoline color value	F_4 hybrids		Hard Federation	Kota	Marquis
	Number	Per cent	Number	Number	Number
0.90	2	6.7			
1.10	0				
1.30	4	13.3			
1.50	2	6.7			
1.70	5	16.7		1	
1.90	9	30.0	1	1	1
2.10	5	16.7	1		1
2.30	3	10.0			
Total	30	100.1	2	2	2

ASH IN FLOUR

The amount of ash in flour is an important factor in the grading and selling of flour. A considerable proportion of the flour trade buys flour on its ash content, as in general it furnishes a basis for judging the extraction in milling. Certain varieties, however, such as Kota, have a relatively high ash as an inherent quality. While a larger quantity of nutritive ash is not objectionable in itself, a variety which normally has a higher ash than the class average is at some disadvantage under present flour standards.

Experiments reported by Clark and Shollenberger (7) have shown Kota to have a significantly higher ash content than that of Marquis. Table IV shows

The data show considerable differences in ash content and more than should be due to differences in milling. There is evidence, therefore, that ash content is inherited as other quantitative characters, and that if sufficient numbers were studied, the mode of its inheritance could be determined and selections made which would have an inherently low ash.

CORRELATION OF CHARACTERS IN THE F_2 AND F_3

An inheritance study of the characters discussed would not be sufficiently inclusive without some knowledge of the effect the more important characters have on each other and on yield. The amount of correlation which is found between the different

TABLE XXXI.—*Segregation of 29 F_4 selections of the Kota-Hard Federation and reciprocal crosses, together with the parents and Marquis, for ash in flour, at Mandan, N. Dak., in 1923*

Ash in flour (class)	F_4 hybrids		Hard Federation	Kota	Marquis
	Number	Percentage	Number	Number	Number
0.55	2	6.9			
0.65	12	41.4		1	
0.75	9	31.0	2	1	
0.85	3	10.3			1
0.95	1	3.4			
1.05	1	3.4			
1.15	1	3.4			
Total	29	99.8	2	2	1

Hard Federation to average less than Marquis in ash content. In the present study of hybrids the ash content of the flour could not be very accurately determined, due to the small amount of grain milled and the nonuniformity of milling. It seemed desirable, however, to determine the ash content of the flour available to see if segregation for ash content appeared to occur in the hybrids. The data obtained are given in Table XXXI.

It will be seen that the ash content is unusually high for both hybrids and varieties. This is largely due to the method of milling, the small amount of wheat used making it difficult to keep part of the germ and shorts from passing into the flour. The wheat also was not scoured, which undoubtedly helped to increase the ash. The higher ash content of Marquis and Hard Federation over that of Kota, may be due in part to their shrunken kernels and low weight, due to injury from stem rust.

characters should serve as an important guide for the making of further selections. Coefficients have been determined for the correlation of several characters with date of heading and yield. Those were calculated by the product moment method for coefficient of correlation. The correlations of height and stem-rust infection with date of heading, and date of heading, height, stem-rust infection, and awn classes with yield, combine the principal data on drought and rust obtained in this study.

Time of maturity is important in determining the ability of a variety to evade or resist drought and rust. It has not been determined how early wheats may mature in the northern spring-wheat area before yield is reduced. High yield may be sacrificed with increasing earliness of varieties. Real resistance to both rust and drought probably is more desirable than earliness. Both might combine to make for maximum success.

DATE OF HEADING AND HEIGHT OF PLANT

The correlation between date of heading and height of plant has been studied in F_2 material grown at Davis, Calif., St. Paul, Minn., and Mandan, N. Dak., in 1922, and F_3 material grown at Mandan, N. Dak., in 1923. The Mandan data are given in Tables XXXII and XXXIII.

height of plant was negative and not important or significant at Davis, Calif., under very favorable conditions, but positive, with increasing importance and significance, under increasingly unfavorable conditions at St. Paul, Minn., and Mandan, N. Dak. In both F_2 and F_3 under droughty conditions at Mandan there was an important and significant correlation between date of

TABLE XXXII.—*Correlation between date of heading and height of plant in F_2 material of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1922*

Height of plant (inches)	Date of heading										Total
	June			July							
	26	28	30	2	4	6	8	10	12	14	
18							1				1
21		1	1		1						3
24		1		2	1	1					5
27		6	7	3	6	1		1			24
30		36	39	18	24	21	6	1			145
33	3	92	118	65	73	47	12	5	1		416
36		64	76	53	65	54	25	7	5	2	351
39	1	5	11	10	23	21	19	7	1	3	101
42			1	3	4	9	1	3			21
45							1				1
Total	4	205	253	154	197	154	65	24	7	5	1,068

$r = 0.250 \pm 0.019$

TABLE XXXIII.—*Correlation between date of heading and height of plant in F_3 material of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1923*

Height of plant (inches)	Date of heading							Total
	June				July			
	20	23	26	29	2	5	8	
18	3	7	9					19
21	24	85	61	4				174
24	23	114	114	10	3	1		265
27	8	45	94	9	8	1	2	167
30		12	39	15	6	3		75
33		2	7	3	6	1		19
36					2			2
Total	58	265	324	41	25	6	2	721

$r = 0.410 \pm 0.021$

A summary of the coefficients obtained is as follows:

F_2, 1922:
- At Davis, Calif.—0.083±0.023
- At St. Paul, Minn. .131± .029
- At Mandan, N. Dak. .250± .019

F_3, 1923:
- At Mandan, N. Dak. .410± .021

It will be noted that in F_2 the correlation between date of heading and height of plant. The early plants more often were found to be short and the late plants to be tall. While the results at Davis indicate that the Mandan results are largely due to environmental conditions, random selection of early plants at Mandan would, to a certain degree, lead to the selection of short plants as well.

DATE OF HEADING AND STEM-RUST INFECTION

The effect of earliness on infection of stem rust is a problem of considerable economic importance. Correlations between date of heading and stem-rust infection have been studied in F_2 material of the Kota-Hard Federation crosses at St. Paul, Minn., and Mandan, N. Dak., in 1922, and F_3 material at Mandan in 1923. The Mandan F_2 data are given in Table XXXIV.

TABLE XXXIV.—*Correlation between date of heading and percentage of stem-rust infection in F_2 material of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1922*

Stem-rust infection (per cent)	Date of heading										Total
	June			July							
	26	28	30	2	4	6	8	10	12	14	
5		4	1	1	1						7
15		42	31	9	12	2	1				97
25	1	30	30	16	13	16	2	1			109
35	1	27	53	24	28	23	6	5	1		168
45		45	60	45	46	36	11	5	1	1	250
55	1	27	33	21	30	22	12	5			151
65	1	26	36	28	48	36	23	7	3	2	210
75		4	5	8	16	12	7		2	2	56
85			4	2	2	6	2	1			17
95					1		1				2
Total	4	205	253	154	197	153	65	24	7	5	1,067

$r=0.311\pm0.019$

The coefficients obtained are summarized as follows:

F_2, 1922:
At St. Paul, Minn. 0.093 ± 0.029
At Mandan, N. Dak. $.311\pm.019$
F_3, 1923:
At Mandan, N. Dak. $.156\pm.025$

A significant positive correlation was found between date of heading and stem-rust infection in three instances. The coefficient was not large at St. Paul, but a fairly high degree of correlation was indicated at Mandan in both years. The wheat in 1922 was later in maturing at Mandan than at St. Paul, due to later seeding and being farther north, and rust may have had as good a chance to infect the early plants as the late ones. On the other hand, there probably was more rust inoculum present in the latter part of the ripening period, so that some of the early plants may have partly escaped. Selections of rust-resistant plants, therefore, must be thoroughly tested to be certain that they are resistant rather than escaping. A few fairly late plants in both F_2 and F_3 appeared resistant at Mandan.

YIELD AND DATE OF HEADING

Productiveness or yield is the desired result of wheat breeding. Other characters are important in so far as they affect yield. Quality is important with high yield. The principal characters previously discussed which could affect production are now studied for correlation with yield.

The effect of time of maturity on yield usually is important in the northern spring-wheat area. The aim of the breeder is to produce just as early a wheat as can be grown without sacrificing productiveness. Data on correlations between date of heading and yield of plant were obtained in F_2 from St. Paul, Minn., and Mandan, N. Dak., in 1922, and in F_3 at Mandan in 1923. The results from Mandan in F_3 are given in Table XXXV.

The coefficients obtained are as follows:

F_2, 1922:
At St. Paul, Minn. $-0.324\pm.026$
At Mandan, N. Dak. $-0.176\pm.020$
F_3, 1923:
At Mandan, N. Dak. $-0.193\pm.024$

A significant negative correlation was found between yield and date of heading under the three conditions. The correlation was most important at St. Paul. At Mandan in both 1922 and 1923 the amount of correlation is not sufficient to be considered very important from a selection standpoint. Early heading selections, however, offer the greater possibilities for high yield.

It seemed desirable to determine the inheritance of date of heading in F_3 in relation to that of F_2. The correlation of 0.758 ± 0.024, obtained from 148 plants and shown in Table XXXVI is very important.

TABLE XXXV.—*Correlation between yield of plant and date of heading of F_3 material of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1923*

Date of heading		Yield of plant, in grams							Total
		0.5	1.5	2.5	3.5	4.5	5.5	6.5	
June	20	------	7	12	21	13	5	------	58
	23	1	21	104	94	33	7	2	262
	26	3	55	129	94	37	5	3	326
	29	1	7	19	11	4	------	------	42
July	2	3	6	5	8	2	1	------	25
	5	2	------	2	1	1	------	------	6
	8	------	1	1	------	------	------	------	2
Total		10	97	272	229	90	18	5	721

$r = -0.193 \pm 0.024$

TABLE XXXVI.—*Correlation between date of heading in F_2 and F_3 of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1922 and 1923*

Date of heading, 1923		Date of heading, 1922								Total
		June		July						
		26. 5	28. 5	0. 5	2. 5	4. 5	6. 5	8. 5	10. 5	
June	20. 5	------	13	------	1	------	------	------	------	14
	22. 5	5	48	11	4	------	------	------	------	68
	24. 5	------	10	10	7	3	1	------	------	31
	26. 5	------	2	7	6	7	2	1	------	25
	28. 5	------	------	------	3	1	1	1	------	6
July	. 5	------	------	------	------	------	------	1	------	1
	2. 5	------	------	------	------	------	------	2	1	3
Total		5	73	28	21	11	4	5	1	148

$r = 0.758 \pm 0.024$

YIELD AND HEIGHT OF PLANT

From a breeding standpoint it is important to determine whether short or tall plants are more productive. Height also is an important economic factor in wheat production. The aim of the breeder is to produce a variety tall enough to be conveniently harvested with a self-binding harvester and still produce maximum yield. Taller varieties have no particular advantage. They are subject to greater injury from storms and use more soil fertility and moisture. Hard Federation often is too short to be conveniently harvested. Kota, on the other hand, is slightly taller than necessary. Height, therefore, should be given careful consideration while making selections of the progeny. But it is first necessary to know the effect of height on yield. Data on correlations between these factors were obtained in F_2 material at St. Paul, Minn., and Mandan, N. Dak., and F_3 material at Mandan. The Mandan F_2 data are presented in Table XXXVII.

TABLE XXXVII.—*Correlation between yield of plant and height of plant in F_2 material of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1922*

Height of plant, inches	Yield of plant in grams													Total
	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	
18	1	------	------	------	------	------	------	------	------	------	------	------	------	1
21	------	1	1	1	------	------	------	------	------	------	------	------	------	3
24	3	2	1	------	1	------	------	------	------	------	------	------	------	7
27	3	15	5	------	1	------	------	------	------	------	------	------	------	24
30	3	25	51	45	20	5	3	2	------	------	------	------	------	154
33	------	26	83	136	102	56	22	6	2	1	1	1	------	436
36	4	14	40	79	97	54	45	16	11	3	2	------	1	376
39	------	------	12	14	32	20	22	10	2	1	------	------	------	113
42	1	------	3	3	7	8	2	1	2	------	------	------	------	27
45	------	------	------	------	------	1	------	------	------	------	------	------	------	1
Total	15	83	196	278	259	155	94	35	17	5	3	1	1	1,142

$r = 0.405 \pm 0.017$

A summary of the coefficients is as follows:

F_2, 1922:
At St. Paul, Minn. 0.348 ± 0.025
At Mandan, N. Dak. $.405 \pm .017$
F_3, 1923:
At Mandan, N. Dak. $.288 \pm .023$

Important and significant correlations between yield and height were obtained. Height appears to be of greater importance than any of the other characters studied. As the amount of the correlation was considerably reduced at Mandan in F_3 as compared with F_2, it is possible that the increase is partly expressive of hybrid vigor. There were no particularly high yielding short plants in F_2. Height had more of an effect on yield at Mandan under droughty conditions than at St. Paul under rather humid conditions. Apparently shortness of plant is not correlated with real drought resistance. The correlations are sufficiently large to justify the selection of tall plants instead of short ones.

It is desirable to determine the inheritance of average height of row in F_3 in comparison with height of F_2 plants at Mandan. The correlation of 0.556 ± 0.038 in 152 F_2 plants, given in Table XXXVIII, is both high and significant.

YIELD AND AWNEDNESS

The effect of the awn or its absence on yield already has been discussed. It was shown that the presence of awns was of some importance under droughty conditions. In addition to determining the mean yield of the plants in each awn class, it seems desirable to determine the correlation between awnedness and yield. The Mandan F_3 data are given in Table XXXIX. The awn classes are (1) awnless, (2) apically-awnletted, (3) awnletted, (4) short-awned, and (5) awned.

TABLE XXXVIII.—*Correlation between height of plant in F_2 and in F_3 material of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1922 and 1923*

Height of row, 1923 (inches)	Height of plant in inches, 1922								Total
	29.5	31.5	33.5	35.5	37.5	39.5	41.5	43.5	
19.5	2	3	1		1				7
21.5	2	2	4	2	1				11
23.5	1	10	12	8	2	1			34
25.5	1	2	8	14	3	3			31
27.5		3	7	8	5	2			25
29.5	1		1	10	10	2	1	1	26
31.5			2	4	4	4	2		16
33.5						2			2
Totals	7	20	35	46	26	14	3	1	152

$r = 0.556 \pm 0.038$

TABLE XXXIX.—*Correlation between yield of plant and awnedness in F_3 material of crosses of Kota and Hard Federation wheats grown at Mandan, N. Dak., in 1923*

Awnedness classes	Yield of plant in grams							Total
	0.5	1.5	2.5	3.5	4.5	5.5	6.5	
1		26	50	31	11	2		120
2	6	40	114	90	37	6	4	297
3	1	12	37	38	9	6	1	104
4		7	31	23	9		1	71
5	3	12	43	48	24	4		134
Total	10	97	275	230	90	18	6	726

$r = 0.104 \pm 0.025$

The coefficients are:

F_2, 1922:
At St. Paul, Minn.. 0.079 ± 0.028
At Mandan, N. Dak. $.051 \pm .021$
F_3, 1923:
At Mandan, N. Dak. $.104 \pm .025$

The correlations are positive but small and hardly significant except in F_3 at Mandan in 1923. The F_2 data show that the presence of awns and their length have but slightly less effect upon the yield under droughty conditions at Mandan than under humid conditions at St. Paul. The F_3 data indicate, although the correlation is small, that preference in the selection of awned plants rather than at random seems justified.

YIELD AND STEM-RUST INFECTION

Stem-rust infection is not necessarily an index to rust damage. The same percentage of stem-rust infection in different years may cause different degrees of damage. An important correlation between rust infection and rust injury may be obtained when rust is the principal limiting yield factor. The years 1922 and 1923 were ones of general rust infection. The damage, however, varied with the time and amount of infection in different localities and with the stage of growth of the wheat plant. A correlation study between the percentages of stem rust and yield of plant furnishes a method of determining rust damage from varying amounts of infection. Data were obtained in this study in F_2 at St. Paul, Minn., and Mandan, N. Dak., and in F_3 at Mandan, N. Dak. The data from F_2 at St. Paul, Minn., in 1922 are given in Table XL.

The coefficients are summarized as follows:

F_2, 1922:
At St. Paul, Minn. -0.082 ± 0.028
At Mandan, N. Dak. $-.009 \pm .020$
F_3, 1923:
At Mandan, N. Dak. $-.042 \pm .025$

The correlations are negative, as expected, but they are small and not significant. The infection of rust that occurred in these experiments, therefore, had little or no effect on yield. An opportunity for selection of resistant plants was offered, however, the importance of which is in no way lessened by the results of these correlations.

It is important to determine how the average infection of F_3 strains is correlated with that of F_2 plants. The data presented in Table XLI show that a large, important, and significant coefficient of 0.674 ± 0.018 was obtained. It may be concluded, therefore, that the resistance to stem rust that occurred is inherited.

TABLE XL.—*Correlation between yield of plant and percentage of stem-rust infection in F_2 material of the crosses of Kota and Hard Federation wheats grown at St. Paul, Minn., in 1922*

Stem-rust infection, per cent	Yield of plant in grams													Total
	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	14.5	15.5	
5	2		1		2	1	2	1						9
15	2	2	7	6	6	4	7	5	1					40
25	4	10	12	15	11	13	10	4	1	3	1	1		85
35	3	14	16	23	14	12	1	2	6	1				92
45	13	27	27	23	25	16	7	7	2		2		1	150
55	1	7	7	9	3	6	6	2	1	1				43
65	3	23	15	15	19	7	8	4	1	1				96
75		4	7	7	4	6	3							31
85	1		4	2	2	2	2	1	1	1				16
95		1	1											2
Total	29	88	97	100	86	67	46	26	13	7	3	1	1	564

$r = -0.082 \pm 0.028$

TABLE XLI.—*Correlation between percentage of stem-rust infection in F_2 and F_3 material of reciprocal crosses of Kota and Hard Federation wheats grown at St. Paul, Minn., and Mandan, N. Dak., in 1922, and at Mandan, N. Dak., in 1923*

Stem-rust infection, 1923 (per cent)	Stem-rust infection, per cent, 1922									Total
	5	15	25	35	45	55	65	75	85	
5		5								5
15	43	74	2	1						120
25	26	91	13	7	6	1	2	1		147
35	6	15	5	8	17	4	6	2		63
45	3	7	9	7	10	6	13	2	3	60
55		1	5	3	1	2	3		2	17
65					4	1	3	2		10
75								1	1	2
Total	78	193	34	26	38	14	27	8	6	424

$r=0.674\pm0.018$

SUMMARY

1. This genetic study was made for the purpose of obtaining resistance to drought and rust in a high-yielding hard red spring wheat of superior milling and bread-making quality.

2. The Kota and Hard Federation varieties were selected as parents for the crosses because they appeared to be the best available material for combining in one study the problems of obtaining resistance to drought and rust, combined with high yield and quality.

3. The material was grown at three points, i. e., Davis, Calif., under ideal conditions, St. Paul, Minn., under conditions of rust prevalence, and Mandan, N. Dak., under droughty conditions.

4. Reciprocal crosses were studied. No important maternal or paternal influence was found although a slight and consistent influence was noted for certain characters. With these, only when the variety with the dominant character was used as the female parent was a fit significantly close to the expected ratio usually obtained.

5. The inheritance of awns was partially explained on a dihybrid Mendelian ratio, in which five classes were studied and the short-awned and awned classes were shown to be recessive to the awnless, apically-awnletted and awnletted classes. Neither the awned nor awnless classes bred true in F_3, and complete homozygosity for these classes could be interpreted only on a multiple-factor hypothesis.

6. Glume color did not appear to be inherited in a monohybrid ratio in F_2, but those data corrected on the basis of F_3, when a part of the recessive white-glumed class segregated, proved that only one genetic factor was involved.

7. The color of the kernel segregated in F_2 in numbers close to the 15:1 ratio. In F_3 the white strains bred true and the red strains bred true or segregated in a 3:1 or 15:1 ratio.

8. Early maturity as determined by date of heading was found to be dominant to late maturity.

9. Tallness of plant appeared to be partially dominant but due principally to heterosis and was easily affected by environmental conditions.

10. Resistance to stem rust proved recessive and in F_2 appeared to occur close to a 1:15 ratio. In F_3, however, not one of nearly 300 resistant F_2 families bred true for resistance, although at St. Paul, Minn., 8.1 per cent of 2,068 F_3 plants were resistant and at Mandan, N. Dak., 23.4 per cent of 6,387 were classed as resistant. Evidence was shown that strains homozygous for resistance could be obtained in F_4.

11. Yield appeared to be due to multiple factors. F_2 hybrids exceeded the parents in variability, but F_3 selections were less variable in comparison with the parents than the F_2 selection.

12. Segregation for quality and quantity of the gluten was shown, by the use of viscosity and crude-protein determinations, to have occurred prior to the F_4.

13. Evidence of segregation for the color and ash of the flour also was indicated among F_4 selections.

14. The amount of correlation between date of heading and height of plant increased with increasingly unfavorable environmental conditions due to rust and drought.

15. A significant positive correlation was found between date of heading and percentage of stem-rust infection; the earlier plants apparently partially escaped rust.

16. Date of heading and yield of plant were negatively correlated, early plants proving more productive. An important correlation was obtained between dates of heading in 1922 and in 1923.

17. Important and significant positive correlations were obtained between yield and height of plant and between heights of plant in 1922 and in 1923.

18. Small positive correlations were found between awn and yield. Differences of 11 to 18 per cent in yield were found in favor of awned over awnless strains.

19. Negative correlations, not important or significant, were obtained between yield of plant and stem-rust infection. An important correlation was obtained between stem-rust infections in 1922 and in 1923, proving that resistance is inherited.

20. Selections have been made from the cross on the basis of the information gained from the investigations. Early, tall, awned, rust-resistant high-yielding plants predominate among the selections. Their quality in F_4 and later generations, based on the quality and quantity of gluten and color and ash of the flour, have been and will be used as a basis for further trial or for elimination. The white types are being grown in the Pacific Coast area and the hard red spring types will be extensively tested under conditions of drought and rust in the northern spring-wheat area.

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EGG AND FIRST-STAGE LARVA OF TARSOSTENUS UNIVITTATUS (ROSSI), A BEETLE PREDACIOUS ON POWDER-POST BEETLES [1]

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INTRODUCTION

The family Cleridae has long been recognized as one of considerable economic importance because of the predacious habits of the beetles, both in the larval and adult stages. Champlain (*1*)[2] states that "they are among the principal predators of wood and bark boring beetles, the adults attacking the adults of the destructive species while the larvae feed upon the eggs and broods in the bark and wood." And he continues:

"Under natural conditions they may be of but normal importance but can be turned to considerable account in control measures with the additional help of man, who can overbalance the natural conditions in favor of the predators by properly conducting control work."

The beetle *Tarsostenus univittatus* (Rossi)[3] is one of the clerid species and, according to Champlain (*1*), principally a predator on powder-post beetles such as Lyctus and Xylobiops (=Sinoxylon) in dry, seasoned wood products.[4] Of this species several living adults were obtained while the writer was supervising a series of experiments which were recently conducted by the Bureau of Entomology in cooperation with the Naval Aircraft Factory at the League Island Navy Yard, Philadelphia, Pa., to determine temperatures fatal to the powder-post beetle *Lyctus planicollis* Le Conte by steaming infested ash and oak lumber in a kiln (*5*). Additional infested ash lumber was sent to the Forest Insect Laboratory, East Falls Church, Va., and several adult beetles were secured from this material to be placed in cages for rearing.

TRANSFORMATION TO ADULTS AND MATING

Most of the beetles probably pass the winter in the larval stage, although a few may pupate before early spring, especially if they are in wood which is kept in a heated building. In December, 1923, samples of infested wood were placed in a heated building and were kept at a temperature of 70° F., or slightly higher. When examined five weeks later, most of the beetles were in the larval stage, but two pupæ and three maturing adults were found. This is in accordance with the statement of Champlain (*1*) that "clerids overwinter sometimes in all stages, and sometimes only in a certain stage. The time of transformation to adults is generally in the spring, but it varies." Thus the first adults emerged from the cages containing the infested lumber during the early part of February.

Probably the adults of *Tarsostenus univittatus* emerge about the same time as those of their host, *Lyctus planicollis*, for Snyder (*3, p. 14, 4*) records that adults of the latter emerged as early as January 12 in a heated building in the vicinity of Washington, D. C., that their general appearance occurs about the middle of April, the maximum emergence from the last of April to the first of June, and the last emergence during the first part of July. All these data coincide with corresponding data for *Tarsostenus univittatus*, as far as observations have been made.

The mating usually occurs shortly after the adults emerge.

OVIPOSITION

Oviposition begins a day or two after the adults emerge from the wood and mate. The beetles were observed to crawl into the entrance galleries of their host, and it is likely that eggs were deposited there. According to Champlain (*1*) "they may be placed in or near the entrance gallery of their

[1] Received for publication April 22, 1924—issued—January, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 51.

[3] Order Coleoptera, family Cleridae.

[4] That the larva does not confine itself to attack on a single host is suggested by the observations of the present writer, who saw a mature larva in rearing crawl over the surface of the wood and enter a gallery of Lyctus from which powderlike borings were being ejected. Altogether, a large percentage of their host must have been killed by the attacks of the beetle, both in the larval and adult stages.

host." In order to observe the process of oviposition three adults, two females and a male, were placed in a small vial which contained a porous stopper. In this way oviposition was observed without much difficulty. The female, before inserting the egg, spends considerable time moving from place to place, with the ovipositor extended, to find a suitable opening. The ovipositor is nearly as long as the body. It terminates in two slender, palpilike processes which are used to locate the desired opening. When such a place has been found the end of the body is placed close to the opening and the ovipositor is inserted into the cavity.

THE EGG

The egg (fig. 1, *a*), in many respects, bears a rather striking resemblance to that of its host, *Lyctus planicollis* (fig. 1, *b*). In fact, when seen in a pore in the wood, it might easily be mistaken for the latter. It (fig. 1, *d*) is elongate, cylindrical, rounded at the posterior end and drawn out into a slender strandlike process at the cephalic end. It is grayish-white in color, somewhat shiny, 0.85 mm. long without the strandlike process, or 1.2 mm. with this process included, and 0.123 mm. in width. The egg of *Lyctus planicollis* is slightly smaller, being 1 mm. long with

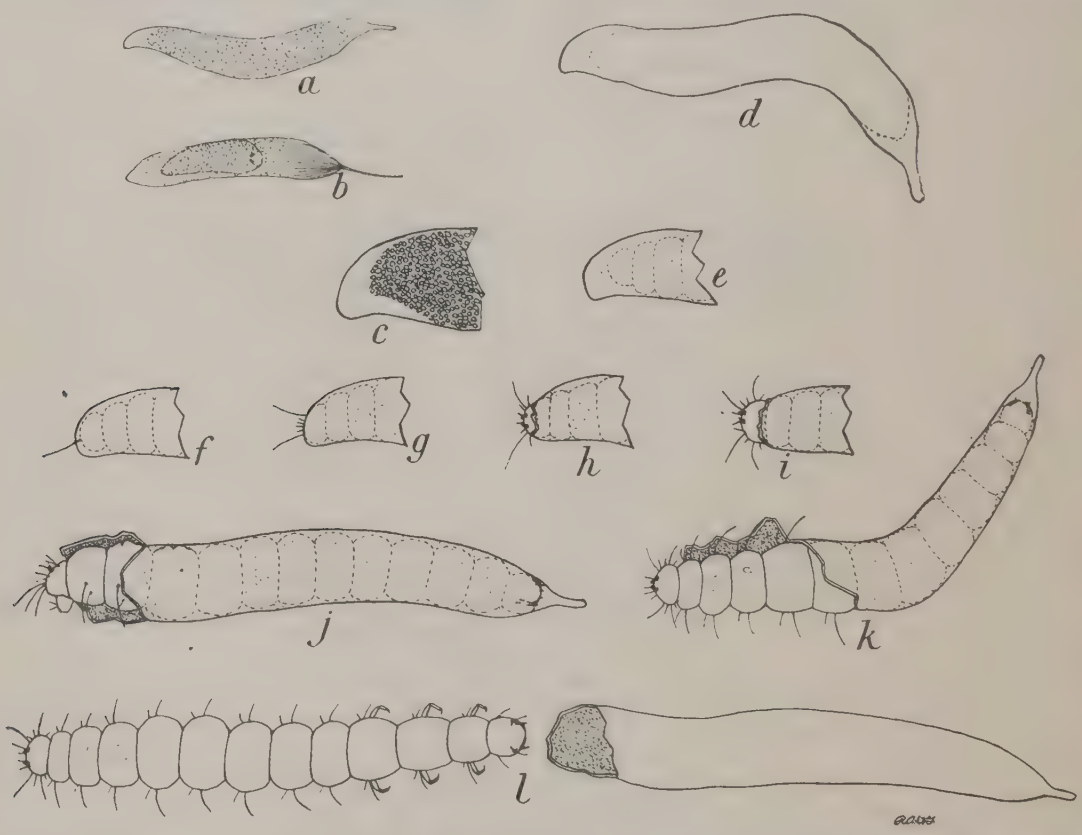

FIG. 1.—*Tarsostenus univittatus: a*, Egg with newly formed embryo; *c*, greatly enlarged view of end of egg, showing granular appearance; *d*, outline of egg, showing strandlike attachment; *e*, part of egg, showing dorsal view of 6th, 7th, 8th, and 9th abdominal segments of larva ready to hatch; *f–k*, larva hatching from egg, liberating first the caudal setae and then the abdominal segments; *l*, larva free from eggshell, and the empty shell. *Lyctus planicollis: b*, Egg with larva ready to hatch, drawn to same scale as *a*.

After a short time, during which a pumping movement was noted in the ovipositor, the egg was inserted into the pore. In one instance seven minutes elapsed before the process of ovipositing was completed. Upon examining the stopper three days later seven eggs were found. They were all inserted in the same cavity, side by side, probably for the simple reason that it was the most favorable place. It is quite likely that under natural conditions from one to three eggs would be deposited in a pore, as is the case with Lyctus.

the process (fig. 1, *b*). The process resembles that on the eggs of *Lyctus planicollis*, and of the bostrychids *Scobicia declivis* Lec. (*2*) and *Xylobiops basilaris* (Say). It has a granular appearance (fig. 1, *c*) like that of Lyctus but differs from the latter by the absence of the longitudinal striae on the end which bears the process. As is the case with the above mentioned eggs which it resembles, the end bearing the strandlike process leaves the ovipositor last.

After the egg is a few days old the formation of the larva within can be

seen, especially when the latter moves. It occupies nearly the entire egg. After a period of incubation of about 10 days the larva becomes quite active and is ready to emerge. It twists and turns, causing the closely-fitting eggshell to follow the movements of its body. At that time, just before hatching, part of the segments assume a violet tint and the mandibles can now be seen.

The process of hatching is a very interesting one and is accomplished in a manner which again is similar to what we find in *Lyctus planicollis* (fig. 1, *e–l*).

When ready to emerge, the larva begins to push against the posterior end of the egg by alternately contracting and expanding its body (fig. 1, *e*). The ninth abdominal segment is armed with stiff setae which are used for piercing the eggshell. First one (fig. 1, *f*) and then several setae may be seen protruding through the end of the shell (fig. 1, *g*). Next, the abdominal segments may be seen gradually working themselves out (fig. 1, *h–k*), until finally the larva frees itself completely and starts to crawl about (fig. 1, *l*).

Under natural conditions the entire process of emerging probably takes less than an hour, but in the laboratory, when the eggs were placed in a plaster cell and little opportunity given for holding the shell in place, it took three hours. When, however, the cephalic end of the shell was held fast the process was completed in considerably less time. From this fact it occurred to the writer that the reason why eggs of this type have the peculiar strandlike process at the cephalic pole is that it enables the adult to attach the strand to the wood in order that the egg can be held in place while the larva emerges. In one instance a larva was unable to emerge from the egg after twisting and turning for a long time, presumably owing to the fact that the egg was not held in place.

THE FIRST-STAGE LARVA

The first-stage larva is armed with setae which are much longer than in the mature specimens.[5] When the larva is in the egg these setae are pressed close to the body, but as soon as the segments are free from the eggshell the setae straighten out. The newly hatched larva is nearly white except for the violet markings on the segments; the ampullae are slightly developed on the terga of the 6th and 7th abdominal segments, a characteristic also of the mature larva. The mandibles, cerci, and claws are lightly chitinized, and four ocelli are present on each side of the head, also as in the mature larva. It has not been possible to find any spiracles.

SUMMARY

Tarsostenus univittatus (Rossi) belongs to the family Cleridae, which is of considerable economic importance because of the predacious habits of the beetles in both the larval and adult stages. Several adults were reared from ash lumber infested with the powder-post beetle *Lyctus planicollis* Le Conte, on which it is known to be predacious.

Under normal conditions the beetles pass the winter in the larval stage and adults emerge in the spring about the time the powder-post beetles appear.

Oviposition begins soon after the adults mate; elongate, cylindrical, grayish-white eggs, which possess a peculiar strand-like process on the cephalic end, are deposited in or near the entrance gallery of their host. The process is probably attached to the wood to hold the eggshell in place while the larva emerges. After a period of incubation of about 10 days, the larva hatches from the egg by backing out, freeing itself by using its long, stiff caudal setae to pierce and break the posterior end of the shell.

The first-stage larva differs from the mature form in that the setae are much longer, but resembles it in having violet-colored markings on its segments; ampullae present on the sixth and seventh terga; the mandibles, claws, and cerci lightly chitinized; and four ocelli on each side of the head.

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[5] A. G. Böving (*1*) has described and figured the mature larvae of this family.

PYTHIUM ROOTLET ROT OF SWEET POTATOES[1]

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The genus Pythium, certain species of which are known to cause "damping-off" of a number of plants in the seedling stage, has never been reported on the roots of sweet potatoes so far as the writer is aware. Sweet potato plants, the rootlets of which were partially decayed by Pythium, were first collected in New Jersey in 1914. Since then it has frequently been observed and collected from various types of hotbeds in New Jersey, Delaware, Maryland, Virginia, and in many of the States in the South and West. It is not unlikely that under suitable environmental conditions the disease may occur wherever sweet potatoes are grown.

Although the Pythium rootlet rot of sweet potatoes occurs principally in the hotbed or seedbed, it has been found on the roots of plants from the time they were set in the field until they were dug. The amount of injury actually caused by the disease is difficult to estimate. Undoubtedly plants having the ends of the smaller roots dead are at a disadvantage and would be slow in starting to grow after being set in the field. Furthermore, observations have shown that infected plants, especially if the soil conditions are unfavorable, remain stunted the entire summer. It is probable that a considerable amount of the loss hitherto attributed to the socalled "sick soils" is actually due to the injury to the root system by Pythium throughout the summer. It is interesting to note in this connection that Rhizoctonia is sometimes associated with the Pythium in the decayed ends of the rootlets.

Pythium rootlet rot has been found in hotbeds or seedbeds prepared by the use of soil which was almost pure sand. It is more prevalent in old beds or beds in which the soil or sand has been used for several years. The amount of infection is apparently increased by an abundance of moisture in the soil. If, on the other hand, the bed later becomes dry, the injury to the plants is increased as a result of the reduction of the root system. Pythium rootlet rot is primarily a disease of the small rootlets, as shown by the accompanying illustration (Pl. 1). The infections take place at the tip ends of the rootlets and from there the fungus grows progressively up the root, killing it for a distance of from ½ to 3 cm. or more from the tip.

In the spring of 1924 twenty-one varieties of sweet potatoes were bedded in soil that had been used for a sweet-potato bed the two preceding years. The soil contained a considerable amount of organic matter in the form of stable manure and decayed roots and vines. When the plants were pulled they were carefully examined and Pythium rootlet rot was found on all the varieties. There was, however, considerable variation in the amount of infection among the different varieties, as shown by the following data: The amount of infection on different varieties of sweet potatoes by the Pythium rootlet rot showed severe infection on six varieties, Big Stem Jersey, Key West, Creola, Little Stem Jersey, Nancy Hall, and Porto Rico; moderate infection on 12 varieties, Red Jersey, Georgia, Triumph, Yellow Belmont, Gold Skin, Dooley, Haiti, Dahomey, Red Brazil, Gen. Grant Vineless, Pierson, and Southern Queen; and slight infection on three varieties, Pumpkin, Yellow Strasburg, and White Yam. This classification is merely an estimate and was determined by a careful examination of the roots of a number of plants. In many cases it was difficult to determine to which group a certain variety belonged, and as a matter of fact, there are several varieties in the "moderately infected" class which are on the border line of either the "severely" or "slightly" infected group.

[1] Received for publication Aug. 30, 1924—issued January, 1925.

Pythium Rootlet Rot of Sweet Potatoes

Plate 1

Note the large number of rootlets that are partially or completely decayed. Infection usually begins at the end of the rootlet and extends progressively up the root

The writer has made no study of the species causing Pythium rootlet rot. The taxonomy of the causal organisms will be handled by Dr. Chas. Drechsler, who is making a monographic study of the genus. Judging from a preliminary study of the large number of cultures Doctor Drechsler has already obtained from the sweet-potato roots, it is not unlikely that two or three species of the *debaryanum* type may be identified.

SUMMARY

The Pythium rootlet rot of sweet potatoes occurs quite commonly in the hotbeds in various parts of the country. The disease apparently has its beginning at the end of the small rootlets and works progressively up the root. Investigations have shown that all 21 varieties tested are susceptible to the disease, but not to the same degree.

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BACTERIAL PUSTULE OF SOYBEAN[1]

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INTRODUCTION

Diseases of the soybean, *Soja max* (L.) Piper, have for several years been the subject of investigation by the writer and his colleagues at the North Carolina Agricultural Experiment Station (*2, 3, 8, 11, 16, 17*).[2] Among those which have been given special study is a leafspot to which the appropriate name "bacterial pustule" has been applied. This leafspot disease was first briefly described (*4*) in 1922 by Florence Hedges. In this preliminary report, Miss Hedges designated the causal organism *Bacterium phaseoli* var. *sojense* and stated that a paper covering the results of her investigations was in preparation (*6*). It might be anticipated that the present report would confirm in all essential features that of Miss Hedges. Should it contribute nothing new to the knowledge of this disease and its causal organism, nevertheless it is believed to have a definite value since the investigations have been conducted entirely independently. It is the writer's purpose, therefore, to embody in this paper a description of the disease, an account of its relation to other soybean leafspots of bacterial origin, studies on its etiology, and on the morphology and physiology of the causal organism.

HISTORY AND DISTRIBUTION

A definite knowledge of the occurrence of this disease dates from Miss Hedges's isolation, from specimens sent from Texas in 1917, of a yellow organism very closely resembling *Bacterium phaseoli* E. F. Smith. However, a bacterial leafspot of soybean which was assumed to be due to *Bacterium phaseoli* was mentioned as long ago as 1904 (*12*). The isolations upon which this report was based were made two years earlier (*13, p. 280*) from diseased soybeans taken from two localities, one near Charleston, S. C., and the other near Washington, D. C. The organism isolated at that time was not proved by inoculation experiments to be pathogenic, and therefore its relation to the leafspot can not now be satisfactorily determined. This problem is further complicated by the fact that other bacterial organisms have recently been found to be pathogenic to soybean. The first of these diseases to be carefully investigated was a bacteriosis (*9*) which manifests itself by the formation of lesions both on stems and pods. Those on the stems are especially characteristic, since they girdle them in a manner suggesting the blackleg disease of potatoes. The pathogen, *Bacillus lathyri* Manns and Taubenhaus, is identical with the one which causes the "streak" disease of sweet pea, *Lathyrus odoratus* L. It is a yellow organism but has very different morphological and cultural characters from the soybean organism under consideration.

As will be shown later, the appearance of bacterial pustule has little in common in any stage of development with that of bacterial blight as described by Miss Coerper (*1*) or by the writer (*16*). The causal organisms identified as *Bacterium glycineum* by the former and as *Bact. sojae* by the latter are both white and are thus easily separable from the pustule organism; but in the case of old lesions caused by *Bact. sojae* the tissues are always occupied also by a yellow, one-flagellate organism (*16*) which may be a source of confusion as to the primary cause. It might be indicated at this point that although the bacterial blight diseases caused by *Bact. glycineum* and *Bact. sojae* are very similar in appearance, the causal organisms are readily distinguishable and specifically distinct, as has been shown by certain cultural studies (*11, 7, 17*).

Mention has been made in several previously published accounts of bacterial diseases of soybeans in the Orient. A bacteriosis has recently been recorded (*10*) by Miura, a Japanese investigator, as occurring in Manchuria. His description of the disease accords with the appearance of

[1] Received for publication Feb. 25, 1924—issued January, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 68.

bacterial blight, and he furthermore expresses the opinion that the disease in Manchuria is the same as that found in America. He also records the occurrence of this bacteriosis in other parts of China and Japan.

In 1921 another Japanese investigator, Takimoto (*14*), published a comprehensive account of his investigations on a bacterial disease of soybeans which he had had under observation since 1914. This disease manifests itself by the formation of numerous small, angular, dark-brown leaf-spots with chlorotic intervening tissues. Lesions may also form along the veins and extend along them making dark streaks. Black, sunken areas form upon both petioles and stems. These symptoms, as he points out, are quite different from bacterial blight in America. The organism which he isolated was proved to be pathogenic not only to soybean in all stages of its growth, but also to Adzuki bean, *Phaseolus angularis* Willd. Takimoto does not assign a name to the bacterium which he had under observation, but does compare it morphologically and physiologically with published accounts of *Bacterium glycineum*, as described by Miss Coerper, *Bact. sojae*, as described by the writer, and *Pesudomonas glycineum*, as described by Nakano,[3] who isolated the organism in 1916 from collections made in Kumamoto prefecture. He concludes that it is most like *Bact. sojae*, but differs from it in the absence of a capsule, in its failure to effect a change in milk, and in its growth in the closed arm of fermentation tubes containing dextrose, saccharose, and mannite. It is doubtful whether these differences would be confirmed were both forms in the hands of one investigator; and it remains for subsequent investigation to determine the identity of the organism of Takimoto and *Bacterium sojae* and whether either or both are identical with *Pseudomonas glycineum* Nakano.[4] Certainly none of these are like the soybean bacterial pustule, as will become apparent when they are compared with the description of the appearance of this disease and with the cultural characters of the causal organism.

Bacterial pustule is known to occur in Texas, Virginia, Kansas, South Carolina, and Louisiana (*3*, *4*). It has been collected from a sufficient number of localities in North Carolina to warrant the belief that it is generally prevalent throughout the State.

APPEARANCE OF THE DISEASE

Soybean pustule has not been observed upon the stems and pods, but upon the foliage only. Soybeans in all stages of growth varying from the seedling stage to mature plants are subject to infection. The disease may appear upon the foliage in any stage of maturity, but reaches its most destructive stage of development at the time when the plants have reached their maximum vegetative growth.

The first indication of the disease is the presence of minute elevations on either or both leaf surfaces (figs. 1 and 2). These elevations themselves are light green in color. Soon a yellowish-green halo forms as a border at the base of each elevation. At this stage the lesions are easily distinguished from those produced by *Bacterium glycineum* and *Bact. sojae*, neither of which causes the formation of pustules, and each of which, especially in the early stages, causes the invaded tissues to be translucent. This is followed by the enlargement of the elevations to very prominent pustular outgrowths often extending above the leaf about twice as high as the thickness of the leaf (fig. 3). These raised portions soon collapse and, together with a portion of the surrounding invaded tissues, become brown. Ultimately, the lesions become angular to irregular reddish-brown areas which vary in size from minute specks to large irregular spots. These large areas arise by the fusion of lesions in case initial lesions are abundant. Lesions in all stages of development occur on the same leaflet. This indicates that the initial infections serve as the source of inoculum for secondary infections.

The general aspect of the pustule disease in the late stages (Pls. 1, 2) is like that produced by the rust fungi, whereas bacterial blight lesions are dark brown to brownish-black in color and tend to break out and fall away, thus making perforations or notches in the leaves.

Nakano's original paper has not been seen by the writer, nor does he have a complete reference to it. These papers were translated by Mr. S. Yamamoto, a special student of textile manufacture at the North Carolina State College of Agriculture and Engineering. Grateful acknowledgment is herewith made of his kindness and courtesy in rendering this service.

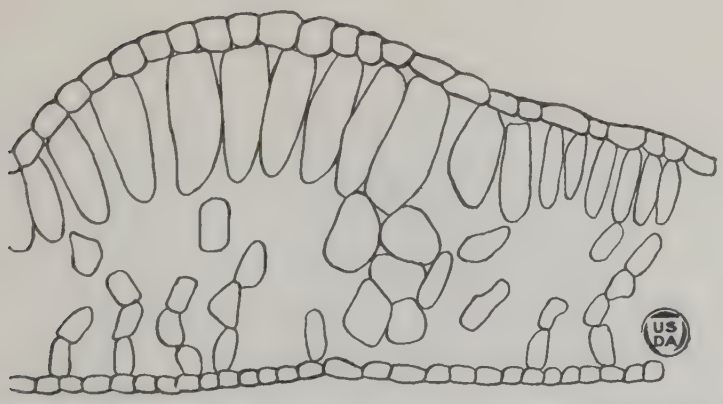


FIG. 1.—Diagram outlined with camera lucida showing beginning of pustule formation on upper leaf surface

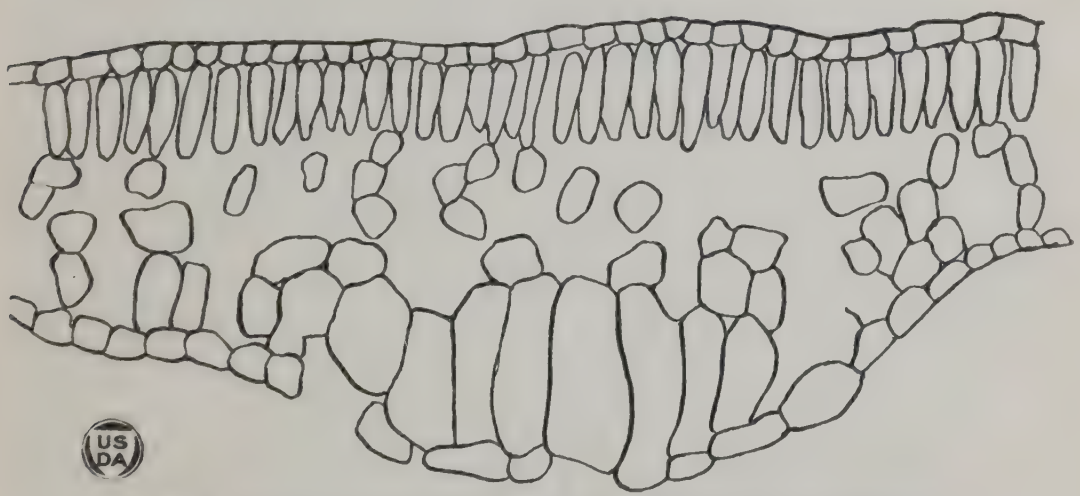


FIG. 2.—Pustule formation involving the tissues of the lower leaf surface

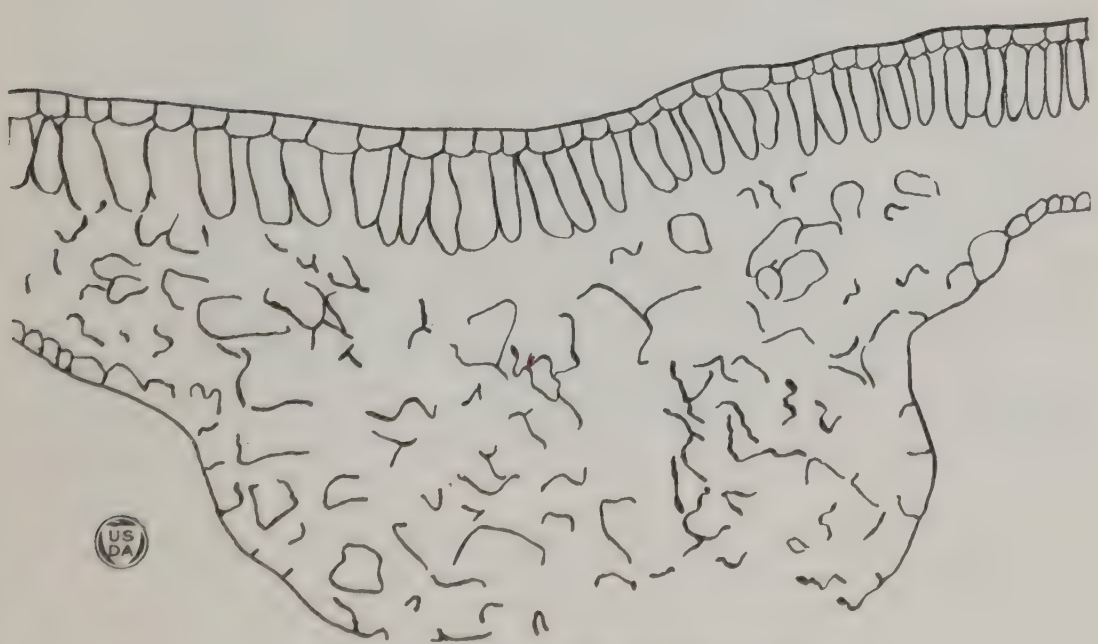
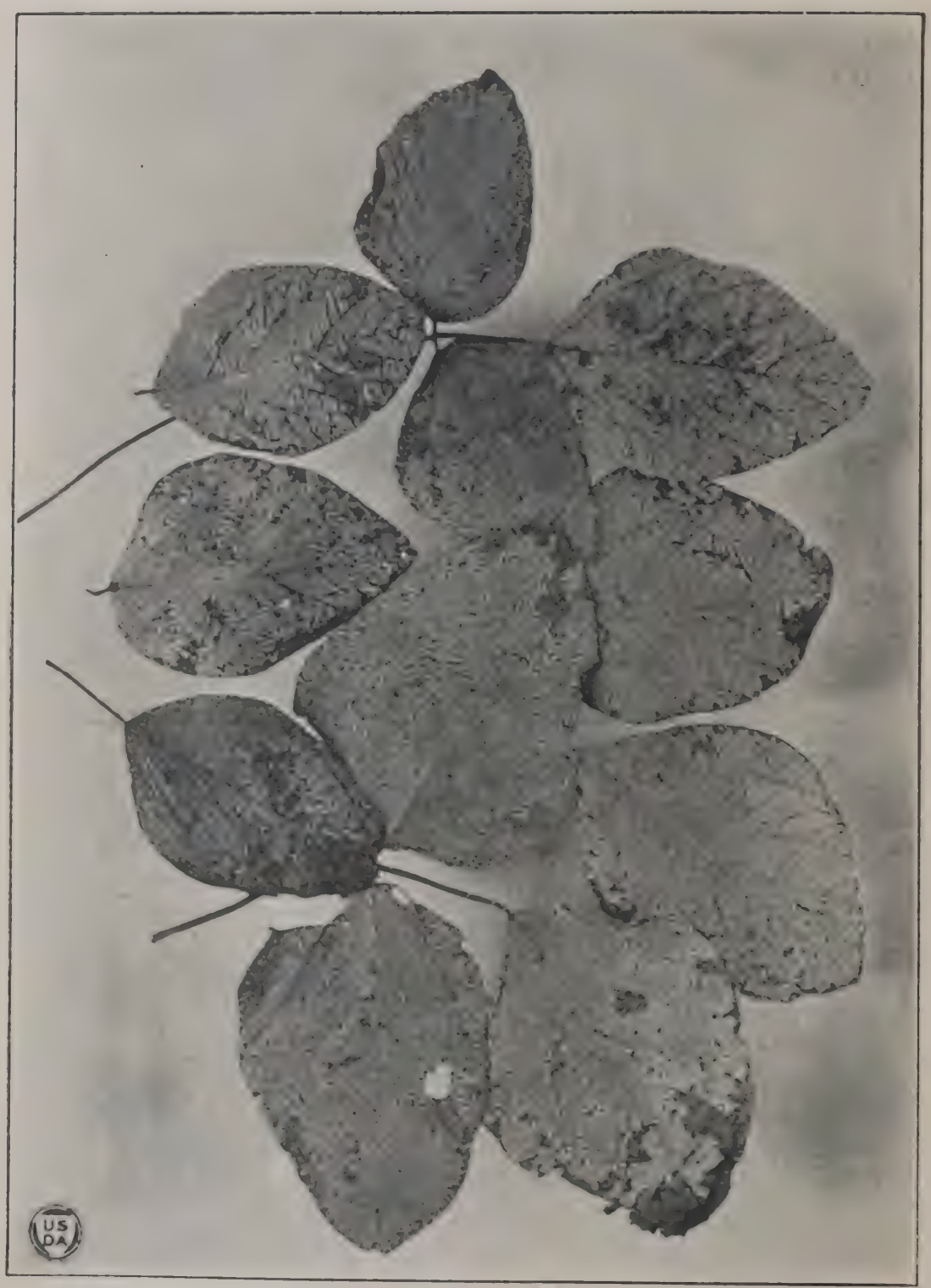


FIG. 3.—A large pustule whose cells have collapsed



Bacterial Pustule of Soybean

Plate 1

Bacterial pustule of soybean showing natural infection in various stages of development.

Bacterial Pustule of Soybean

Plate 2

Leaves, natural size, affected with bacterial pustule

CAUSAL ORGANISM

ISOLATION

In preparations made by macerating lesions in a drop of water, the invaded tissues were found, upon microscopic examination, to be teeming with bacteria. These bacteria swarm out in such abundance as to cloud the water and form a turbid suspension, a phenomenon characteristic of diseases of bacterial origin. If an inoculum is prepared by direct maceration of young lesions in sterile water and a loopful of this inoculum is transferred to an agar plate and spread over its surface with a zigzag stroke, discrete colonies of the pathogen form near the end of the stroke. These can then be selected for transfer to tube cultures. A considerable number of strains were isolated by this procedure during the course of the present investigation. Subsequent comparative study of these strains to determine their cultural characters and their ability to infect soybean showed them all to be identical. Since it was apparent from the preliminary studies that the soybean pustule organism is closely related to *Bact. phaseoli*, several strains were compared with *Bact. phaseoli* isolated from pod lesions of Lima bean, *Phaseolus lunatus* L., and garden bean, *Phaseolus vulgaris* L.; and with a strain of bean blight isolated by Miss Hedges. The following parallel studies of the morphology and physiology of all strains both from soybean and from Phaseolus show that all are practically identical.

MORPHOLOGY

VEGETATIVE CELLS.—The primary cause of soybean bacterial pustule is a yellow rod-shaped organism with rounded ends. When taken directly from lesions it is found to occur singly or in pairs, but tends in bouillon to form short chains. The organism stains readily with all of the more common bacteriological stains. When stained from 24-hour potato-agar cultures with carbol fuchsin, the elements are 1.3 to 2×0.6 to 0.75 μ. Such preparations, too, show the presence of investing material, as is manifest also with Welch's capsule stain; but this envelope can not be interpreted as indicating a well-defined capsule. When stained by Gram's method, the organism is decolorized. Endospores and marked involution forms have not been noted.

When the organism from young lesions, from bouillon cultures, or from 24-hour agar cultures is examined for motility, it is seen to possess the power of rather active locomotion. That this movement is due to the presence of a polar flagellum about twice the length of the cell has been demonstrated by several modifications of the method of Loeffler.

CULTURAL CHARACTERS.—The various media employed in the cultural studies were prepared by the methods employed by the writer and his associates in their studies on the physiology of certain plant pathogenic bacteria (*17*). The nutrient broths contained 1 per cent Difco peptone and 0.3 per cent Liebig's beef extract; the nutrient agars the same, with the addition of 1.8 per cent of bacto-agar. The hydrion concentration of cooled media was measured colorimetrically and the media were not heated after adjustment of reaction. The carbon compounds were sterilized in distilled water and added with aseptic precautions to cooled media. The cultures were incubated at room temperatures which approximated 20° to 25° C.

NUTRIENT AGAR.—The colonies may appear in agar plates within 24 hours, but are not prominent, nor do they show the characteristic yellow color until they are 48 hours old. They are circular in outline with entire margins and a glistening surface but with no surface markings. Their consistency varies from nonviscid to slightly viscid. In potato agar or other nutrient agar containing 3 per cent or more of agar, the colonies are convex with internal markings (Pl. 3). With media of a low degree of viscosity, the colonies are flat and spreading with no striking internal convolutions. In agar slants the growth is filiform, spreading at the base of the slant, with an entire or somewhat contoured margin, glistening and translucent. No odor is developed and the agar does not become pigmented.

POTATO CYLINDERS.—On steamed potato cylinders, growth is first manifest by a faint yellowish streak. This becomes abundant within 24 to 36 hours, is spreading, yellow, and has a striking whitish zone along the border of the growth. Within six to eight

Bacterial Pustule of Soybean

Plate 3

Bacterium phaseoli var. *sojense* in plate cultures showing difference in appearance due to difference in viscosity of agar. The upper culture contains 1.8 per cent agar, the lower contains 5 per cent agar. All other conditions are identical.

days the cylinder will have collapsed, and when this collapsed tissue is tested with Lugol's solution the starch will be found to have disappeared, showing that the pathogen possesses strong diastatic properties. An ocular demonstration of the ability of the pustule-forming bacterium to hydrolyze starch may be made by growth in plate culture on beef extract agar plus 0.2 per cent of soluble starch. If the plates are flooded with iodine solution after a week's incubation, a broad clear zone surrounding the colonies will indicate the area in which the starch has been destroyed by enzym activity.

MILK.—Plain sterilized milk becomes separated into whey and curd, and the curd is slowly digested. The first evidence of these enzymatic activities is the appearance of a thin layer of clear whey just at the surface and below the pseudozoogloeal surface growth. As the curd forms, it slowly settles. Cultures three weeks old will have cleared with at most a small quantity of curd at the bottom of the culture tube.

LITMUS MILK.—Lavender-colored litmus milk undergoes the same type of clearing and separation into whey and curd as does plain milk. Little or no free acid is formed, or at least is not formed in sufficient quantity to change the color of the indicator.

CARBON METABOLISM.—In the preliminary tests on the fermentative ability of the soybean pustule organism in which only the common sugars were employed, it was apparent that it could not by this means be separated from *Bacterium phaseoli*. Accordingly, use was made in addition of certain rare sugars which have been successfully employed in distinguishing *Bact. glycineum* and *Bact. sojae* (*11*) and also *Bact. sojae* and *Bact. trifoliorum* (*7*). Bouillon to which was added sufficient of the carbon compounds to make a 1 per cent solution was employed to follow the progressive changes in hydrion concentration as an index to fermentative activity. The sugar was added to cooled, sterile bouillon flasked in convenient quantities and was tubed with aseptic precautions in previously sterilized test tubes. All were made from the same stock bouillon and a considerable number of cultures of each strain were made on the same day. This made possible the employment of several tubes of each strain at each reading at each of the consecutive intervals. The results of these fermentation tests are assembled in Tables I to IV.

TABLE I.—*Fermentation of various carbon compounds by the organisms from soybean pustule (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.0	7.4	7.6
Saccharose	7.0	7.0	7.0	7.4	7.6
Lactose	7.0	7.0	7.0	7.2	7.4
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.2	7.6	7.8
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.6	7.8
Dextrin	7.0	7.0	7.0	7.4	7.6
Salicin	7.0	7.0	7.0	7.4	7.6
Mannitol	7.0	7.0	7.0	7.6	7.8
Inulin	7.0	7.0	7.2	7.6	7.8
Galactose	7.0	7.0	7.2	7.4	7.8

TABLE II.—*Fermentation of the same various carbon compounds by Bact. phaseoli from snap bean (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.2	7.2	7.4
Saccharose	7.0	7.0	7.2	7.2	7.4
Lactose	7.0	7.0	7.2	7.6	7.6
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.2	7.4	7.6
Arabinose	7.0	7.0	7.2	7.4	7.6
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.4	7.6
Dextrin	7.0	7.0	7.2	7.4	7.6
Salicin	7.0	7.0	7.2	7.4	7.6
Mannitol	7.0	7.0	7.2	7.4	7.6
Inulin	7.0	7.0	7.2	7.4	7.6
Galactose	7.0	7.0	7.2	7.4	7.6

TABLE III.—*Fermentation of various carbon compounds by Bact. phaseoli from Lima bean (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.2	7.4	7.6
Saccharose	7.0	7.0	7.2	7.4	7.6
Lactose	7.0	7.0	7.2	7.4	7.6
Maltose	7.0	7.0	7.2	7.4	7.6
Glycerin	7.0	7.0	7.2	7.4	7.6
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.4	7.8
Dextrin	7.0	7.0	7.2	7.4	7.6
Salicin	7.0	7.0	7.2	7.4	7.6
Mannitol	7.0	7.0	7.2	7.4	7.8
Inulin	7.0	7.0	7.2	7.4	7.8
Galactose	7.0	7.0	7.2	7.2	7.4

TABLE IV.—*Fermentation of various carbon compounds by strain of Bact. phaseoli from Washington, D. C. (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.0	7.2	7.4
Saccharose	7.0	7.0	7.0	7.2	7.4
Lactose	7.0	7.0	7.0	7.2	7.4
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.0	7.4	7.4
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.0	7.2	7.4
Rhamnose	7.0	7.0	7.0	7.2	7.4
Dextrin	7.0	7.0	7.0	7.2	7.6
Salicin	7.0	7.0	7.0	7.2	7.6
Mannitol	7.0	7.0	7.0	7.2	7.4
Inulin	7.0	7.0	7.0	7.4	7.6
Galactose	7.0	7.0	7.0	7.2	7.4

It is evident from an analysis of the data presented in these tables that the soybean pustule organism and the several strains of *Bact. phaseoli* are identical so far as concerns their inability to utilize any of these carbons as the source of energy and that they can not therefore be separated on their fermentation relations. The increase in alkalinity, as appears in all cultures with all sugars, develops from the decomposition of the proteins, as shown by growth in plain bouillon.

GAS PRODUCTION.—These tests were conducted by using fermentation tubes filled with portions of the same solutions as were used in the fermentation tests. These tubes were sterilized prior to filling and were incubated for 48 hours after being filled to determine their freedom from contamination. They were then inoculated in sets of four with each of the several strains. No gas was developed in the case of any of the 13 carbon compounds, and growth was sharply limited to the open arm in all media with each of the strains.

NITROGEN METABOLISM.—Unfortunately, no considerable significance has been attached to the nitrogen metabolism of bacteria in relation to the determination of species. This matter has been based largely upon their fermentative ability. It would appear that plant pathogenic bacteria, especially those which attack few or no carbon compounds, as is the case with those under consideration, might be separated upon the basis of differences in nitrogen metabolism were methods of study known. The writer's attempts in this direction have been limited to the employment of a few nitrogenous compounds, added as nutrients to a stock synthetic agar. This agar was prepared according to the following formula: Distilled water, 1,000 cc.; magnesium sulphate, 0.5 gm.; dipotassium hydrogen phosphate, 1.0

gm.; potassium chloride, 0.5 gm.; ferrous sulphate, 0.01 gm.; agar, 20 gm.

DIGESTION OF CASEIN.—Evidence in addition to that obtained in milk cultures of the ability of the organism from soybeans and *Bact. phaseoli* to digest casein was obtained by adding 1 per cent casein to the stock agar in poured plate cultures. After a week's incubation wide halos, in which the casein was entirely dissolved, had formed around the colonies, thus demonstrating the ability of these organisms to form erepsin.

DIGESTION OF ASPARAGIN.—Stock agar plus 1 per cent of asparagin was used in these tests. The indicator consisted of a sufficient quantity of 4 per cent solution of rosolic acid, and sufficient NaOH was added to give the medium a decided orange color and a reaction of about P_H 6.0. In poured plate cultures incubated for about 10 days, the orange color gave way to a beautiful brilliant red. This change begins with a halo around each colony and comes to involve the entire plate. The change in color is due to the liberation of ammonia in the decomposition of asparagin as a result of the activity of the enzym amidase.

DIGESTION OF SERUM.—Blood serum added to stock agar in poured plate cultures was planted with the several strains of bacteria. This medium in cultures 7 to 10 days old serves as a satisfactory means of demonstrating the ability of these organisms to liquefy blood serum.

LIQUEFACTION OF GELATIN.—Growth in stab cultures on gelatin is slow, but within a period of two weeks the gelatin to a depth of about a centimeter will have become liquefied. Liquefaction begins as an infundibuliform area.

REDUCTION OF NITRATES.—Nitrate broth consisting of 1 per cent peptone, 0.3 per cent beef extract, and 0.1 per cent potassium nitrate supports abundant growth. No indication of nitrites was secured when the tests at appropriate intervals were made with sulphanilic-acid solution or with naphthylamine acetate solution.

INDOL PRODUCTION.—No indication of indol was secured by either the Salkowski, Vanilin, or Ehrlich test.

THERMAL DEATH-POINT.—In determining the thermal death-point, tubes of bouillon P_H 6.6 were inoculated from vigorously growing bouillon cultures and subjected for 10 minutes in the usual manner to various trial temperatures. As a result of these tests, and under these conditions, the thermal death-point was found to be 50°C.

RESISTANCE TO DESICCATION

An inoculum of the soybean organism from cultures on potato cylinders 48 hours old was suspended in sterile water and drops of this suspension were transferred to sterile cover glasses kept in sterile Petri dishes. After desiccation at laboratory temperatures certain of these cover glasses were, at definite intervals, inserted into tubes of nutrient broth. Growth appeared after 18 days' desiccation, hence the organism may be regarded as very resistant to drying.

PATHOGENICITY

Only a few pathogenicity trials were made. Pure cultures from young transfers on potato agar were suspended in sterile water, and this bacterial suspension was applied with an atomizer. When inoculations were made late in the afternoon and the plants were covered until the following morning with bell jars, a large number of centers of infection developed. The first evidence of infection was noted within four to six days, appearing as tiny elevations. Within a week later these had developed into typical lesions. From these the original organism was reisolated in trials with several of the strains. Plants in all stages of development from those with the first pair of true leaves to plants with mature foliage are subject to infection with pure cultures. Both garden beans and Lima beans have been inoculated in the same manner, and in some cases with a portion of the same inoculum; but no evidences of infection have been observed on these inoculated plants.

When *Bacterium phaseoli* was employed as an inoculum on Phaseolus and on soybeans, an abundance of typical bean-blight lesions developed upon both the foliage and pods of Lima bean and of garden bean but no evidence of pathogenicity to soybeans was noted.

Miss Hedges has reported (*4*, *5*) the production of infection on soybeans and several varieties of garden beans following inoculation with pure cultures of the soybean pustule organism. The spots on Phaseolus were like those caused by *Bact. phaseoli* with no evidence of pustule formation such as occurs on soybean. She furthermore has found that *Bact. phaseoli* from Phaseolus is only weakly pathogenic to soybean.

The differences in regard to cross inoculation are no doubt due to conditions of inoculation. This opin-

ion is supported by the fact that it has been impossible to separate the soybean organism from bean blight morphologically and culturally. It seems advisable, therefore, to regard the soybean organism as a variety of *Bact. phaseoli*.

RELATION OF PARASITE TO HOST TISSUE

Lesions which were appropriately fixed in alcohol, embedded, sectioned, and stained with alcoholic methylene blue were employed in histological studies. Entrance of the parasite is very manifestly effected through the stomates which occur on both leaf surfaces, as evidenced by the fact that the substomatal chamber and the intercellular spaces of the tissues immediately surrounding the stomata in the case of young lesions are densely filled with bacteria (fig. 4). In the older lesions, after the host cells have collapsed, the bacteria are not confined to the intercellular spaces, but occur within the cell cavities. These phenomena are entirely in accord with those known to occur in the case of nearly all plant bacterial pathogens which invade parenchyma. So far as pustule formation is concerned, however, the writer's observations are not in agreement with the statement made by Miss Hedges (*4*) that the pustules show both hypertrophy and hyperplasia. The preparations in hand show that these elevations arise wholly from hypertrophy and without hyperplasia. Any or all of the tissues, epidermal, palisade parenchyma and mesophyll, may be involved. The physiological phenomena which attend these hypertrophic changes are dependent no doubt upon certain enzymatic activities which involve both the cell walls and the cell contents. The parasite and host relationship and thus the proximate cause of pustule formation is believed to be analagous to that which obtains in the case of citrous canker caused by *Pseudomonas citri* (*15*). In the case of this organism evidence has been advanced that both cytolytic and diastatic enzyms are secreted. Through their activity these modify the osmotic properties of invaded cells and thereby are responsible for their enlargement.

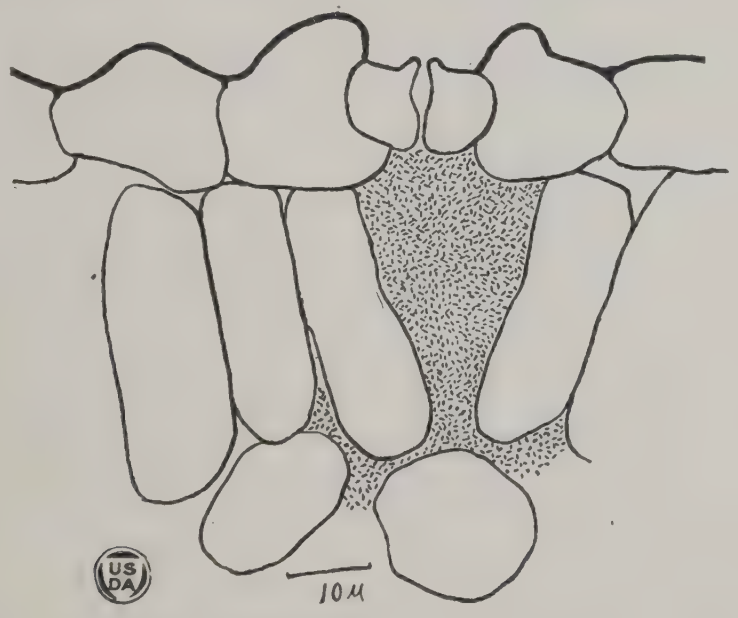


FIG. 4.—Invasion of substomatal cavity by *Bact. phaseoli* var. *sojense*

RÉSUMÉ OF SALIENT CHARACTERS

On the basis of the foregoing studies, *Bact. phaseoli* var. *sojense* is a rod-shaped organism occurring singly, in pairs, or catenulately. The cells measure 1.3 to 2.0×0.6 to .75 μ, are motile by means of a single polar flagellum, possess no well-defined capsule nor endospores, are strictly aerobic and Gram-negative. Colonies on nutrient agar are circular, raised, smooth, shiny, yellow and have an entire or slightly lobed margin. The organism is capable of liquefying gelatin and blood serum, digesting casein and asparagin, is strongly diastatic, very resistant to drying, nonnitrate reducing, and forms neither acid nor gas from the various carbon compounds. Its thermal death-point is approximately 50° C. It has not been possible in culture to distinguish it from *Bact. phaseoli*. According to the 1920 descriptive chart

of the Society of American Bacteriologists its group number is 5322–31135–1333.

SUMMARY

(1) The present investigation concerns a leafspot disease of soybean called bacterial pustule, which is generally prevalent in North Carolina. It is known to occur also in Texas, Louisiana, South Carolina, Virginia, and Kansas.

(2) The disease is distinct from bacterial blight and from the diseases of bacterial origin which have been described in the Orient.

(3) Bacterial pustule appears to be confined to the foliage. Lesions are manifested by the presence of pustular outgrowths on either or both leaf surfaces. They are light green at first, but at maturity collapse and become dry and reddish brown, and the tissues surrounding the lesions become chlorotic.

(4) The disease is caused by an organism to which the name *Bacterium phaseoli* var. *sojense* was first tentatively given by Miss Hedges. This organism is herein fully described and found to be morphologically and culturally indistinguishable from *Bact. phaseoli* E. F. Smith. It forms yellowish colonies on nutrient agar, is flagellate, is unable to utilize any of the carbon compounds tested except starch but can utilize a number of proteins including gelatin, casein, blood serum, and asparagin. According to the descriptive chart of 1920 of the American Society of Bacteriologists, its group number is 5322–31135–1333.

(5) When the organism in watery suspension is applied to uninjured soybean, foliage infection is evident within four to six days. Under the same conditions of inoculation garden beans and Lima beans failed to become infected.

(6) The parasite gains entrance through the stomates and passes thence into the intercellular spaces. The pustules arise by hypertrophic changes of any of the parenchymatous tissues.

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THE CHEMICAL EXAMINATION OF VARIOUS PEAT MATERIALS BY MEANS OF FOOD STUFF ANALYSES [1]

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INTRODUCTION

Several studies have been made in the past few years, and are still being conducted, with the object of accumulating information concerning fundamental differences in peat lands and their relation to agriculture. Prominent among these studies are those dealing with type profiles of peat deposits; that is, with the number of different kinds of peat, their position and arrangement relative to one another, in deposits in different parts of the country. Results thus far secured lead to the conclusion that any adequate description of peat land and the several layers composing it must recognize three fundamental facts: (1) The differences in type of peat and the profile position of the materials; (2) the water level in its relation to the surface zone of oxidation and the lower zone of reducing action; (3) nature of the mineral subsoil and the water supply affecting the quantity and character of salts, such as lime, iron, sulphur, etc.

CHEMICAL ANALYSES OF VARIOUS KINDS OF PEAT

The following study was undertaken to obtain data on the differences in the principal groups of organic compounds of several types of peat layers and to establish, if possible, standards for grading types of peat as to stage of decomposition and relative agricultural value. In determining the relative usefulness of different kinds of peat, for example, to microorganisms and higher plants, it is necessary to find adequate means of indicating the amount of different organic substances which the various layers of peat actually contain. It is obviously important to know what variation may be expected in the layers of peat in different regions of the country in varying stages of decomposition, and at different depths. Likewise it is evident that the composition of any layer of peat, whether stated in terms of calories, of soluble organic material, or of insoluble residue, contributes toward a basis for judgment and for securing standards of value for commercial peat products.

The simplest means for compiling chemical composition records are analyses as made for crops and feeding stuffs, in accordance with official methods (1)[2]. The results obtained in regard to the amount of crude protein, crude fiber, nitrogen-free extract, and fat present in different peats can be readily compared with those derived from analyses of plants and feeding stuffs.

The results reported here [3] indicate that data of this kind are probably fully as important as those secured by other means for determining changes and losses of organic constituents in peat materials varying with composition, stage of decay, and depth below the surface. In many particulars, however, the grouping under these terms of organic substances in different kinds of peat is unsatisfactory. The method does not give a sufficiently forceful illustration of the available organic compounds in plant remains now stored as layers of peat. It would, therefore, be preferable to differentiate between the several nitrogenous substances, carbohydrates and fats, and substitute a more accurate, though necessarily more complicated classification.

Tables I and II represent the chemical composition of samples from typical layers of peat obtained over a great portion of this country. A number of these types of peat have been illustrated in a recent paper (*9*).

[1] Received for publication Apr. 9, 1924—issued January, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 81–83.

[3] The analytical work on this series of peat samples was done by G. L. Bidwell of the Cattle Food and Grain Investigation Laboratory, Bureau of Chemistry, U. S. Department of Agriculture.

TABLE I.—*Foodstuff analyses of various peat materials*

Type of peat	Locality	Condition	Depth below surface	Inorganic matter		Organic matter			
				Water	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Fat
I. SEDIMENTARY			*Ft.*						
Colloidal	Fremont, Ind	Gelatinous	4	2.58	65.48	9.63	12.21	9.81	0.29
Macerated	Lake Okeechobee, Fla.	Finely divided	1	9.81	32.67	15.75	14.19	27.43	.15
Do	Middle River, Calif.	Coarsely divided	2	9.40	22.39	13.25	15.25	38.48	1.23
II. FIBROUS									
Sedge (*Carex* sp.) peat	Chelsea, Mich	Coarsely fibrous	4	7.71	4.97	17.81	30.16	36.51	2.84
Do	Corona, Minn	Finely fibrous	6	8.99	18.67	13.63	23.82	33.79	1.10
Do	Deerfield, Wis	Fibrous, partly carbonized.	3	6.14	10.88	18.69	20.89	42.46	.94
Sedge (*Cladium* sp.)	Okeelanta, Fla	Coarsely fibrous	2	11.24	7.20	19.63	33.20	28.48	.25
Sedge (*Scirpus* sp.)	Middle River, Calif.	Fibrous, partly decomposed.	2	9.19	16.06	14.56	16.80	42.52	.87
Reed (*Phragmites* sp.)	Plymouth, Ohio	Fibrous	3	8.74	3.42	13.13	21.82	52.18	.71
Do	do	"Muck," well decomposed.	0	10.97	16.65	17.69	9.05	45.20	.44
Do	Wood County, Wis.	Fibrous, slightly decomposed.	2½	8.29	5.31	11.69	17.78	53.15	3.78
Brown moss (*Hypnaceae* sp.)	Phillips, Wis	Fibrous, partly decomposed.	5	9.84	19.77	9.81	11.71	46.63	2.24
Do	Algoma, Miss	Fibrous	2	10.76	7.54	13.94	23.19	43.82	.75
Bog Moss (*Sphagnum* sp.)	Fairbanks, Alaska	Coarsely fibrous	1	9.66	3.80	4.03	41.86	38.99	1.66
Do	Culver, Minn	Fibrous	1	9.82	2.54	2.75	40.69	42.73	1.47
Do	Calais, Me	Fibrous, partly decomposed.	2	9.43	1.07	4.38	30.21	52.94	1.97
III. WOODY									
Heath shrub and sedge	Charlevoix, Mich.	Partly decomposed	2	8.17	2.98	9.94	20.81	54.94	3.16
Forest peat (coniferous)	Margie, Minn	do	3½	10.81	6.38	10.13	24.67	46.08	1.93
Do	Charlevoix, Mich.	do	1	13.97	18.89	10.44	17.55	37.66	1.49
Forest peat (mixed)	Winamac, Ind	Largely decomposed.	2½	10.82	22.84	10.69	15.70	39.51	.44

TABLE II.—*Relation of carbonaceous to nitrogenous material in different types of peat examined by means of foodstuff analyses—calculated on a moisture and ash free basis*

Type of peat	Locality	Depth below surface	Organic matter				Ratio of C to N [a]
			Crude protein	Fiber	Nitrogen-free extract	Fat	
I. SEDIMENTARY		*Feet*					
Colloidal	Freemont, Ind	4	30.15	38.23	30.71	0.91	2.2
Macerated	Lake Okeechobee, Fla.	1	27.38	24.67	47.69	.26	2.6
Do	Middle River, Calif.	2	19.43	22.36	56.41	1.80	4.0
II. FIBROUS							
Sedge (*Carex* sp.)	Chelsea, Mich	4	20.40	34.54	41.81	3.25	3.7
Do	Corona, Minn	6	18.84	32.93	46.71	1.52	4.2
Do	Deerfield, Wis	3	22.52	25.17	51.18	1.13	3.1
Sedge (*Cladium* sp.)	Okeelanta, Fla	2	24.07	40.71	34.91	.31	3.0
Sedge (*Scirpus* sp.)	Middle River, Calif.	2	19.48	22.47	56.89	1.16	4.0
Reed (*Phragmites* sp.)	Plymouth, Ohio	3	14.95	24.84	59.40	.81	5.6
Reed "Muck" (*Phragmites* sp.)	do	0	24.44	12.50	62.45	.61	3.0
Reed (*Phragmites* sp.)	Wood County, Wis.	2½	13.53	20.58	61.51	4.38	6.0
Brown moss (*Hypnaceae*)	Phillips, Wis	5	13.94	16.64	66.24	3.18	5.9
Do	Algoma, Minn	2	17.06	28.38	53.64	.92	4.8
Bog moss (*Sphagnum* sp.)	Fairbanks, Alaska	1	4.66	48.37	45.05	1.92	20.0
Do	Culver, Minn	1	3.14	46.43	48.75	1.68	30.3
Do	Calais, Me	2	4.89	33.75	59.15	2.21	19.0
III. WOODY							
Heath shrub and sedge	Charlevoix, Mich.	2	11.19	23.41	61.84	3.56	7.6
Forest peat (coniferous)	Margie, Minn	3½	12.23	29.79	55.65	2.33	6.9
Do	Charlevoix, Mich.	1	15.55	26.14	56.09	2.22	5.2
Forest peat (mixed)	Winamac, Ind	2½	16.11	23.67	59.56	.66	5.1

[a] The terms C and N in this column are used as a numerical expression of the relation of crude fiber and nitrogen-free extract to crude protein.

At this time only a few of the principal groups of plant products can be considered, and but a few examples of the chief kinds of peat material have been examined. This new matter, however, offers a basis for reviewing the results of some of the more recent peat investigations made by various workers. Although the aims of sectional workers are divergent, their contributions are necessarily interdependent. Tentatively these and the results of the present series of analyses are summarized and discussed in relation to (1) nitrogenous substances in various types of peat; (2) nonnitrogenous substances, including crude fiber and nitrogen-free extract; (3) ether-alcohol extract; and (4) mineral matter.

The search for new facts concerning the physical, chemical, and bacteriological qualities of the various layers of peat should take into account all the basic facts and conclusions which are of importance to practical workers in peat land utilization.

NITROGENOUS SUBSTANCES IN DIFFERENT PEAT MATERIALS

The nitrogenous substances in peat materials are of interest agriculturally and industrially in several ways. One to five per cent of organic nitrogen may be found in peat, but little of this is available as plant food. Jodidi (*21*), Stutzer (*50*), and others have shown that nitrates are not present in freshly dug peat materials. The quantity of nitrogen as ammonia is very small, ranging from a few thousandths to a few hundredths of 1 per cent of nitrogen. The water solubility of peat nitrogen varies within wide limits. More nitrogen can be extracted from a sample of peat in a finely divided state than from a coarse fibrous or woody type of material. Peat digested at higher temperature and pressure in an autoclave gives a larger amount of water-soluble nitrogen. The amounts and concentration of acid or alkaline reagents, the duration of digestion, as well as other methods of manipulation in the laboratory have an influence upon the percentage of nitrogenous bodies that can be extracted from peat materials. The relative value of peat materials containing high amounts of these compounds depends on the means by which the nitrogenous substances in peat layers can be extracted by distillation and obtained as ammonia, or otherwise can be converted into an available source of plant food. It has long been known that a composting procedure of some kind must be adopted to make peat nitrogen easily accessible either for crops grown upon peat land, or for plants grown on mineral soils to which peat is applied for the production of humus.

The nitrogenous substances in peat materials are very complex, because in addition to the nitrogenous constituents of plants, bacteria, and molds, the layers of peat also contain the remains of animals of various kinds. On account of their great variety and complexity little more than a beginning has been made in determining the differences in the composition of nitrogenous bodies in organic materials. The extreme complexity and variety of the organic compounds in soils has been demonstrated mainly through the work of Schreiner and his collaborators (*46*). Only recently, however, investigations have been undertaken upon the nature of these compounds in the different kinds of peat and upon the variations in the availability of their nitrogen.

In peat the crude protein may consist of a large proportion of inert substances of animal origin. A very considerable part of this fraction in sedimentary types of peat is frequently due to the admixture of chitinous compounds derived from certain seeds, fungi, insects, the egg cases and skeletal portions of various crustaceous, and other forms of plankton life. Birge and Juday (*3, p. 31*) report that crude fiber derived from plankton crustacea yielded 5.9 to 6.2 per cent of nitrogen. This material is relatively unhydrolyzable and has a low decomposition value.

In air-dried peat materials, the crude protein constitutes from 2.75 per cent to 19.63 per cent of the organic matter (Table I). The results show clearly that the range of variation is largest in the fibrous group of peat materials. Sphagnum peats yield the smallest percentage, varying from a little more than 2 to about 4.5 per cent. In comparison with these the sedge and reed peats yielded over 11 to 19.63 per cent. On the other hand, woody and sedimentary types of peat average only about 11 per cent.

Table II shows the variations in crude protein when stated on a moisture and ash-free basis. The maximum of 30.15 per cent is found in the gelatinous peat from Fremont, Ind., and the minimum percentage in the sphagnum peats from Culver, Minn., Fairbanks, Alaska, and Calais, Me. Peats of the sedge, reed, and woody types contain from 12 to 24 per cent of crude protein. Taken as a whole, the results show distinct dif-

ferences in the percentage of crude protein among the three chief groups of peat materials. There is a considerable increase in the amount of crude protein, from 14.95 to 24.44 per cent, in the reed peat from Ohio in its raw condition, and after it has been transformed into "muck" by cultivation.

In the present state of our knowledge concerning the particular type of nitrogenous compounds in different types of peat the term crude protein is inadequate to cope with the accumulated facts. For reasons given in the following section not much significance can be attached to the amounts of crude protein obtained, at least in so far as the agricultural use of this material is concerned.

The proteins and amides are subdivided on the basis of the nature of their products of hydrolysis and upon the solubilities of the individual compounds. For a rough division the nitrogen insoluble in lead-acetate solution is considered protein nitrogen. The nitrogen that is evolved as ammonia upon treatment with acid, subsequently distilled by potash but not by calcium carbonate, is considered as amide nitrogen. However, it is not yet well understood to what extent these terms may be applied to the fractions obtained from the different kinds of peat.

A review dealing with the study of proteins and amides in soils and peat is available in the literature given below; hence it is unnecessary to go into any detail here. As early as 1844 Mulder (*34*), continuing the investigations made by Einhof, Sprengel, and others, pointed out that the organic nitrogenous material in soils is largely of protein origin. Later, Detmer (*10*) observed that a considerable portion of this nitrogen could be liberated by adding nitrous acid. Jodidi (*21*) determined the relative quantities of mono- and diamino-acid nitrogen together with the nitrogen obtained as ammonia from a fibrous sedge peat. Similarly Suzuki (*51*) reported that he isolated such compounds in a material derived from peat. Robinson (*38*) found that treatment of peat with acid, and lengthening the time of hydrolysis increased the amount of amino nitrogen, until it reached a constant maximum. Robinson concluded that in comparison with ordinary proteins the amino group of peat nitrogen did not exist free but in some resistant form of combination which differed radically from the acid amides. Gortner (*16*), working with three different but undetermined kinds of peat, obtained a maximum of 7.5 per cent of total nitrogen soluble in 1 per cent hydrochloric acid, and an average of 3.78 per cent; in five samples of unchanged vegetable materials (oat straw, alfalfa hay, oak leaves, sweet-fern leaves and grasses from a peat bog) he found a maximum of 34.58 per cent and an average of 20.10 per cent. According to Gortner, these findings would indicate that in the humification of vegetable materials there is a decrease in total nitrogen soluble in very dilute acids. Later, Morrow and Gortner (*33*) studied the amounts of the various forms of nitrogen isolated from samples of sphagnum peat and from material to be identified, it seems, as sedge and sedimentary types of peat. It is quite possible that the material designated by the authors as "sphagnum peat and subsoil" would be found to consist of a layer of sphagnum on sedge peat, and that the "calcareous black peat" is of pond-formed origin, while the analyzed "muck" is sedge peat under cultivation.

Miller and Robinson (*39*) divided the acid soluble nitrogen in peat materials into ammonia nitrogen, acid amide nitrogen, and amino nitrogen, calling attention to the fact that the largest two fractions are the acid amides and the monoamino acids. They further point out that, contrary to a statement frequently found in the literature, there is no regular increase in nitrogen content with depth or age of the peat material, the fluctuations being determined rather by differences in the botanical composition of the various layers of peat. These results are in accordance with those of Zailer and Wilk (*53*), Bersch (*2*), Minssen (*32*), and others, outlined in Bulletin 802 (*8*).

In regard to the decomposition of peat materials, the results of both American and Eurasian peat investigations are of much interest. They indicate that during the accumulation of plant remains as peat, the protein bodies are profoundly altered. Continued submersion of layers of peat in water renders difficult the conversion of their nitrogen into ammonia. A high water level precludes both oxidation of the compounds involved and favorable bacterial activity. When submerged, the transition of the plant remains to peat materials is usually a rapid one; this also accounts for the greater stability of peat nitrogen. Many of these substances remain unavailable even after the water-logged layers of peat have been drained and aerated. Some of the nitrogen may be split off by heating, steaming, or treating the

peat material with acids or alkalis. But at present this method is not an economical one. By composting peat with manure, sewage, or other plant and animal waste products, however, a relatively large portion of the peat nitrogen may be made soluble, and may be mineralized by the action of bacteria and fungi. Thus far the bacteriology of this process has received but scant attention.

NONNITROGENOUS SUBSTANCES IN VARIOUS KINDS OF PEAT

Carbohydrates constitute the major portion of the organic material in plants as well as in peat, and they form one of the most important sources of energy for microorganisms. The carbohydrates include the lignose or woody portion of plant products, the cellulose with related forms, the pentoses corresponding to sugars, the pentosans usually associated with starches and celluloses, and many nonnitrogenous acids. Although certain groups of plants produce a characteristic diversity of carbohydrates, yet all these substances are closely related chemically, being composed of the same elements.

Due to the comparatively large amount of carbonaceous compounds in layers of peat, the fermentation and carbonization of this type of raw organic materials is advocated. Carbonized peat acts as an absorbent for uncrystallized residues from beet and cane-sugar refining. Charred material of this kind has been used in the United States as an ingredient in the preparation of commercial feed with cottonseed meal and molasses, thus permitting the feeding of large quantities of these materials to livestock and poultry. It is claimed that properly prepared, carbonized peat contains valuable nutritive ingredients and acts as a corrective and preventive in livestock diseases. The investigations of Kellner (*23*), Goy (*17*), Stutzer (*50*), Godden (*14*), and others have shown that as a matter of fact peat does not have any particular nutritive value when used as a basis for stock feed, whether in raw condition, treated with acid, or carbonized.

The possibility of using dried and charred peat as a diluent with mineral fertilizers or with tankage, and the use of peat innoculated with bacteria in compounding of commercial fertilizers is also a matter of considerable interest. Much depends upon adequate general information, and upon devising new and more effecient methods for determining the grade and value of the several kinds of peat material used in such products.

The course of decomposition of peat layers through bacterial action is greatly influenced by the relative content and quality of nonnitrogenous and nitrogenous substances. In regard to the carbon-nitrogen ratio Zailer and Wilk (*53*) and Bersch (*2*) have found that the relation of these two elements does not change with the depth of a peat deposit, but varies with the botanical composition of the peat-forming plant remains. The conclusion to be drawn from the results reported in Tables II and III is in accordance with these authors. As in other cases, discussed below, peat deposits with an irregular and complex pedomorphic profile show no regularity between carbon-nitrogen value and depth or age of the peat material. However, when a deposit is examined which has the same botanical composition throughout, the investigations show a tendency toward the attainment of a maximum value of carbon and nitrogen, and a minimum for oxygen and hydrogen.

Little is known regarding the complex of factors which would enable one to designate peat materials as superior, good, fair, or poor in quality. In this country it has not been determined experimentally whether peat materials with a wide carbon-nitrogen ratio are more or less valuable than those with a narrow ratio of carbonaceous and nitrogenous material. Information presented by Löhnis (*26*) makes it very probable that peat materials with comparatively large quantities of carbonaceous compounds and relatively low amounts of nitrogen are not as advantageous as those in which the relation of nitrogen to carbon is a narrower one.

The data in Table II indicate that under similar conditions peat layers derived from sedges, reeds, hypnum mosses, and woody plant remains in which the average relation of C:N is equal to 6 or 10:1 are of a better grade and would make better "muck" during soil-forming processes than the layers of sphagnum peat in which the relation of nonnitrogenous to nitrogenous matter is as 20:1. These results also show that the sedimentary, finely divided pond-formed layers of peat possess a very narrow range of about 2C:1N. The largest variation is in the fibrous group of peat materials.

These facts support the information conveyed in Bulletin 802 (*8*) relating to the economic rank or value and the

relative agricultural possibilities of the several types of peat in this country. The presence of a narrow carbon-nitrogen ratio in sedimentary kinds of peat, as in the case of intensively cultivated fibrous and woody "muck" soils, may be considered to show not only advanced decomposition but also a deficiency in fresh carbonaceous materials. To restore a more balanced ratio, an application of green manure might be advantageous. On the other hand, organic materials of a wide ratio, such as represented by the sphagnum type of peat, may not favor decomposition processes or an increase in nitrogen except upon the addition of peat materials of much narrower carbon-nitrogen ratio or upon the application of liquid stable manure, tankage, sewage sludge, or mineral fertilizers supplying more soluble nitrogen.

Since carbonaceous substances constitute by far the greater part of the organic matter in peat, the relative importance of some of the different forms of nonnitrogenous compounds deserves consideration. In the present method of classification the carbohydrates are grouped as crude fiber and nitrogen-free extract.

Crude fiber is the term applied to the more resistant lignose and cellulose substances of plant products. It is determined by digesting a sample successively with weak acid and alkali (1.25 per cent) and by washing out the soluble material. What remains undissolved, free from ash, is termed crude fiber.[4] The analytical data obtained on various types of air-dried peat are shown in Table I. A very considerable portion of the fibrous light types of peat derived from mosses, sedges, and reeds, constitutes crude fiber. The largest percentage is 41.86, found in the sphagnum peat from Alaska. Partially disintegrated phases of the fibrous group of peat contain relatively less fiber. The well-decomposed and cultivated "muck" derived from reed peat yields the minimum of 9.05 per cent. The decrease noticeable in the amount of crude fiber varies with the degree of decomposition rather than with age or depth of the peat layer below the surface. To a great extent, the plant remains in shrub and forest peat also are fiber; homogeneous structureless peat material of sedimentary origin likewise responds to this test. Finely divided, gelatinous peat and the coarsely macerated water-laid organic matter furnish between 12 to 15 per cent of crude fiber. The differences between the quantity of crude fiber are of similar magnitude when expressed in terms of ash and moisture-free materials (Table II). The amount of crude fiber ranges from a minimum of 12.5 per cent in the reed "muck" from Ohio, to a maximum of 48.37 per cent in the Alaska sphagnum peat. In general, the percentage is comparatively lower the greater the degree of decomposition.

Chemical analyses show that pentosan forms a considerable portion of the fiber component; it is usually associated with cellulose. Peat materials yield measurable amounts of furfural when distilled with hydrochloric acid, the furfural being collected as the phloroglucide, and the result calculated to pentosan. This constituent belongs to a complex less stable towards decay, diminishing in percentage progressively as the peat material becomes chemically altered. Von Feilitzen and Tollens (*12*) have shown that pentosans and methyl pentosans are quite generally present in layers of peat and that sphagnum mosses or the upper and less decomposed horizons of sphagnum peat contained as much as 14.7 per cent and 12.75 per cent of pentosans, respectively. As a rule these high percentages were accompanied by relatively high percentages of nitrogen. Leavitt (*25*) obtained pentosans in appreciable amounts from a peat material of the Florida Everglades. Since the amount of pentosan present in most plant products is roughly in proportion to the amount of fiber, von Feilitzen and Tollens (*12*) suggested the possibility of employing the pentosan determination as a means for indicating quantitatively the degree of decomposition of peat materials. In the light of Rose and Lisse's work (*40*) and the investigation extended by Gorbenko (*15*), it may now be accepted that the assumption is justified by proof. According to Street (*49*), it is possible to adopt the phloroglucin method in use for the determination of pentosans in cattle feed, as a means of detecting the presence of peat in commercial fertilizers.

The outstanding components of crude fiber are lignose and cellulose substances. Both classes of material have been found in peat by various investigators.

The lignose group of organic materials consists of a base of true cellulose

[4] The filtering of the fiber proved to be very difficult on account of the gelatinous nature of the treated peat sample.

which in the course of time has been altered by the addition either of encrusting substances or of chemically combined noncellulose constituents. Hence the lignose materials in plant remains are not definite compounds like cellulose, but vary in composition owing to the thickening of the cellulose surface by adsorption of different substances from the sap in trees, shrubs, and other woody plants. The composition of lignin has not been definitely determined; it is more resistant to decay and difficult to hydrolyze. Lignin is obtained by treating wood with strong hydrochloric acid in accordance with the method of Willstätter and Zechmeister (*52*). The insoluble portion contains a methoxyl-yielding complex when treated with concentrated hydrochloric acid in the manner originally described by Zeisel (*54*). Methoxyl (CH_3O) has been determined in the wood of a large number of trees by Ritter and Fleck (*37*), while Shorey and Lathrop (*48*) found it present in the organic matter of widely different mineral soils. But the fact that its occurrence bears a relation to lignocellulose tissue indicates that the quantity of methoxyl in the organic matter of mineral soils and in that of different layers of peat varies with respect to the kind of vegetation that is the source of the lignose compounds, and with their persistence during the process of decay. As the decomposition of woody material progresses, and the less stable cellulose disappears, an increase will become apparent in the methoxyl content. In the Velen peat deposit, which has been studied by Fischer and his coworkers (*13*), the quantity of methoxyl in sphagnum moss peat is less than that in the woody layers of peat; the sedimentary organic material from greater depths shows a corresponding increase in the methoxyl content of the portion insoluble in acid. The results recorded by Fischer are important also in that they show by the action of solvents a general agreement between the botanical composition of peat materials and their content in bitumen and in alkali-soluble material. The German investigators point out that the methoxyl content does not vary necessarily with increasing depth but that it fluctuates, exhibiting, however, a tendency toward the attainment of a maximum value in woody plant remains. The results confirm the earlier work of Rose and Lisse (*40*).

Cellulose is very common in the plants from which peat is derived. There are a great many varieties of cellulose, and hence the term must be taken as denoting a group (*6*). Evidence of the existence of cellulose in peat materials has been obtained by von Feilitzen and Tollens (*12*). Comparative analysis showed that the cellulose content decreases during decomposition. The samples contained from 6.64 to 15.37 per cent of cellulose, those taken from layers below the surface of a peat deposit yielding the smaller percentage. Sphagnum moss contained from 20.8 to 21.42 per cent of cellulose.

It has long been known that cellulose could be broken down into reducing sugar by treatment with acids. Keppeler (*24*) using a strong solution of sulphuric acid (72 per cent), developed a method of hydrolysis for determining the degree of decomposition of peat layers. Tests made with this method showed that sphagnum peat of recent origin is greatly hydrolyzed and hence has a very low degree of decomposition, scarcely exceeding 25 per cent. Samples of sphagnum peat of older origin were less strongly hydrolyzed, the degree of decomposition varying between 70.9 and 78.8 per cent. Tests of the profile of a peat deposit indicated that the degrees of decomposition increased with the depth below the surface of the deposit. A comparison of the Keppeler method with that of von Feilitzen (*12*) leads Gorbenko (*15*) to the conclusion that on peat materials of Russian deposits the Keppeler method is inaccurate and too complicated, probably owing to the differences in the profiles of the peat deposits and to the difficulty of removing the product of hydrolysis.

More recently, Schneider and Schellenberg (*44*) reported briefly on the action of different solvents for cellulose in peat. These authors observed that samples of various kinds of peat treated with carbon bisulphide and sodium hydroxide (Xanthogenic acid), or with the ammoniacal cupric oxide (Schweitzer's reagent) show a moderate solvent effect. The yields of insoluble residues are not materially less than those obtained by treatment with alkali alone. The cellulose of peat materials appears to be more resistant in alkalies, but the proportion of organic material soluble in alkali, referred to as humic acids, is much larger in the deeper and older layers of peat (*45*). The action of reagents such as ammonia and sodium hydroxide is noteworthy in that it points more clearly to the differences connected with the botanical composition of the respective layers of peat. It would seem, therefore, that the

differing facility of hydrolysis in the peat materials is to be related to the different origin and character of the carbonaceous material, rather than to depth or any subsequent alterations of the peat material in the deposit.

Results obtained by H. M. Cooper of the Pittsburgh, Pa., Experiment Station of the United States Bureau of Mines in the analysis of samples collected in cooperation with the Michigan Land Economic Survey in Charlevoix County, are presented in Table III, IV, and V. The peat materials were taken from three different type-profile deposits. Each of these had the same botanical composition throughout, so that any marked changes in the material due to variation in the composition of the peat-forming plants were eliminated. The carbonization and extraction tests on the three principal groups of peat were carried to 550° C. maximum temperature. The samples were carbonized in air-dried condition and crushed to pass a 60-mesh sieve. The gas obtained was practically free from light oils. The work so far carried on with type materials respectively from sedimentary, fibrous, and woody peat indicates clearly a definite variation in the character and the amounts of the thermal products from carbonization, corresponding with the differences in the origin and the botanic composition of the peat material, obtained at whatever depth.

Aside from the great interest which a high content of fiber and of the cellulose group of substances in peat may have for the industries, their dominant value agriculturally is due to the fact that carbonaceous compounds are the food supply and the source of energy for microorganisms in the soil.

TABLE III.—*Proximate and elementary analysis on different peat materials, air-dried, from Charlevoix, Mich.*

Components	Sedimentary aquatic peat	Fibrous sedge peat	Woody forest peat
Moisture	6.1	7.5	12.0
Ash	21.6	4.6	19.2
Volatile matter	57.9	61.1	47.8
Fixed carbon	14.4	26.6	21.0
Carbon	40.0	51.7	39.6
Nitrogen	3.4	2.2	1.9
Oxygen	28.0	34.7	33.5
Hydrogen	5.6	6.0	4.9
Sulphur	1.4	.8	.9
Calories	4,044	4,972	3,583

TABLE IV.—*Carbonization and extraction tests on various types of peat material from Charlevoix, Mich.*

Type of peat	Dry tar and water-soluble salts	Water	Ammonia	Gas, cubic feet per ton	Residue	Alcohol soluble	Ether soluble
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*
Sedimentary	12.5	24.3		3,560	45.7	0.42	0.18
Fibrous sedge	9.4	25.9		4,595	41.3	1.38	.71
Woody forest	4.7	23.6	0.075	4,760	51.6	.84	.38

TABLE V.—*Analysis of gas obtained from carbonization tests on different types of peat from Charlevoix, Mich.*

Type of peat	Unsaturated hydrocarbons	Oxygen	Hydrogen	Nitrogen	Carbon monoxide	Carbon dioxide	Methane	Ethane
Sedimentary	4.5	0.5	14.3	4.1	8.2	39.9	19.5	9.0
Fibrous sedge	2.2	.6	11.0	4.3	13.3	39.7	25.6	3.0
Woody forest	1.8	1.3	14.4	11.6	8.3	44.6	14.3	3.6

Crop production on peat deposits is dependent on processes leading to the decomposition of carbonaceous materials. Hence, the biological phenomena which transfer cellulose to soluble compounds are obviously of fundamental importance. But investigations upon quality and quantity of available carbonaceous compounds in different types of peat are still problematical. It is certain that bacteria as well as fungi are present in definite horizons of peat deposits, and that both act differently in the several kinds of peat. Microorganisms which make use directly of cellulose or live on its decomposition products may depress or favor the accumulation of nitrogen to a marked degree. An accurate knowledge of the biochemical value of the various layers of peat from different depths and stages of decomposition, and the soluble products formed from them under water-logged conditions and during soil-forming processes, is of great practical value. The rapidity with which the cellulose component becomes decomposed may indicate the differences in the fertility of peat land. The investigations undertaken by Kellerman and his collaborators (*22*, *29*) on the destruction of cellulose by bacteria and filamentous fungi have done much to stimulate this phase of the peat problem. The work of Löhnis and his students (*26*, *28*, *41*) to determine the nutritive value of peat humus for Azotobacter, the studies of Hutchinson and Clayton (*20*), Christensen (*5*) and others, have demonstrated the widespread occurrence of cellulose-destroying bacteria and that they usually act symbiotically, utilizing probably intermediate products. It would be desirable in this connection to ascertain more accurately the degree of utilization of specific peat materials by different bacteria and fungi. Based on observations made in the field, certain fungi are unique in the way their mycelium incloses completely portions of fibrous or woody layers of peat at depths from 2 to 4 feet below the surface, and form clusters of fruiting bodies in the space formerly occupied by the peat material. No reference covering these observations has been found in the literature.

In view of the fact that the present classification of carbonaceous substances in peat materials includes intermediate products of decomposition in the nitrogen-free extract, it is important to determine the amount and the distribution of this fraction in different layers of peat.

The nitrogen-free extract, as given in Tables I and II, composes all carbonaceous residues free from fiber, crude protein, fatty substances and ash. The quantity of this fraction is determined by difference. The nitrogen-free extract embraces a great variety of soluble organic substances. In the analyses of crops and feeding stuffs this fraction represents the starches, sugars, pentoses, nonnitrogenous acids and similar compounds, including glucosides, pectins, and tannins. It is unfortunate that for this group of substances at present peat investigators must rely upon figures obtained "by difference." The extent of the analytical error as well as the actual composition of this fraction in the different types of peat remain unknown.

The analyses in Table I show that the nitrogen-free extract in air-dry peat materials varies from 9.81 per cent, in the Fremont, Ind., gelatinous peat, to 54.94 per cent in the heath-shrub peat with fibrous sedge admixture obtained from Charlevoix, Mich. Most samples contain 30 to 40 per cent. The two samples from the Florida Everglades show a relatively low percentage, approximately 27.8 per cent. The small proportion of nitrogen-free extract in the Florida fibrous sedge peat, and in certain other peat samples, is doubtless to be explained by the loss of dispersed and colloid organic material in the progressive leaching characteristic of humid regions. Calculated on a moisture and ash-free basis this fraction of organic matter ranges from a minimum of about 30.71 per cent in the colloidal peat from Fremont, Ind., and of 34.91 per cent in the leached fibrous peat from Florida, to a maximum of 66.24 per cent in the hypnum peat from Phillips, Wis. In the majority of peat samples, however, it amounts to 40 and 50 per cent. Again the quantity of the nitrogen-free extract exhibits a tendency to vary not with depth below the surface, but with the composition of the plant remains and their relative stage in chemical alteration. As shown in Tables I and II, the more decomposed reed, sedge, and sphagnum types of peat, irrespective of their age or position in the profile of a deposit, yield more than 50 per cent of substances classed as nitrogen-free extract, though some of the component materials are undoubtedly lost by leaching. Coarsely fibrous sedge and sphagnum peat contain much less of this fraction. Woody and sedimentary peat material, high in fiber, also supply only moderate amounts. The results suggest strongly that the state of decomposition does not run parallel to the depth at which a peat material is found below the surface, but that an approximate correlation exists be-

tween the botanical composition of the layer of peat and its relative stage of decay. If this conclusion is further substantiated, the quantitative estimation of the nitrogen-free extract in different layers of peat materials should be of special significance and value. The quantity of this fraction of organic material must influence the character of cultural operations and the rate of growth of crops in the peat-soil horizons through which roots can penetrate. It is quite reasonable to assume that the nonnitrogenous components in the separate layers or horizons in peat deposits may be responsible for the differences in the water-holding capacity of peat materials and in the absorption power for certain mineral salts and fertilizers. Changes of this kind may likewise contribute to layers with varying salinity, and to the stratification of acid and alkaline reaction in certain peat-land profiles. The horizons and pockets of well-preserved shells of mollusks (*9*, *Pl. 1, 4*) in submersed fibrous and sedimentary types of peat, reacting acid to litmus, appear to indicate that the organic acids present are only slightly soluble in water and act upon calcareous materials with great difficulty.

Undoubtedly a more detailed examination of peat layers in regard to their nitrogen-free extract would shed much light upon their microbiological character and upon the biochemical changes going on during the humification of peat materials. Under natural conditions of high-water level, that is, when reduction prevails, the solubility of the substances grouped into this fraction is apparent in the color imparted to the streams which emerge from marshes, bogs, and swamp forests. The content of water-soluble organic matter varies probably with the pedomorphic structure of the peat deposit and with the relative capacity for chemical alterations of the several layers of peat. The color of the solution obtained from freshly dug peat samples varies with different stages of decomposition, ranging from a light straw-yellow to a deep brown. Freezing and heating or steaming different peat materials are accompanied by marked changes in the content of dispersoid and colloid organic material. Studies are reported by Ostwald (*36*) and by Melin and Odén (*30*), which indicate the possibility of using a colorimetric method for investigations of this kind.

Frequently organic substances in peat, forming suspensions and colloidal solutions with water, have a strongly acid reaction and absorb oxygen from the air. Little is known as to their direct effects on plants, although by former investigations (*7*) it was shown that such aqueous peat extracts may contain organic substances of harmful character. A tendency was observed to inhibit or retard and dwarf the growth of higher plants as well as of aerobic microorganisms. The question is still an open one, however, whether some of the organic substances can be absorbed by plants and can be used as sources of carbon.

The dilute alkali extract of peat materials and of the organic matter in mineral soils has been the object of numerous studies on organic decomposition. It is not the purpose of the present paper to review or to discuss the voluminous literature dealing with this phase of the problem. The results of investigations on humus, humic acids, on theories of adsorption and related phenomena have been comprehensively reviewed by Löhnis (*26*), Ehrenberg (*11*), Hoering (*19*), and more recently by Odén (*35*). But with reference to the several kinds of peat and the marked stratigraphic differences in peat deposits, the situation remains problematical. Neither the relation of microorganisms to the formation of muck and humus is understood, nor is the chemical composition of the organic compounds in the nitrogen-free extract of specific types of muck and peat a well-known subject. It should be highly instructive to determine whether the increase in nitrogen-free extract is due largely to the decomposition of cellulose and lignin complexes or to that of other plant products. Attempts should be made therefore leading to a more detailed analysis and to the isolation of compounds from the nitrogen-free extract. Indeed the change toward the formation of such substances is so marked that a comparative cellulose and lignin determination of various peat materials undergoing decay might well serve to throw further light upon the problem of humus.

THE ETHER EXTRACT IN DIFFERENT PEAT MATERIALS

Ether-alcohol extract of peat materials contains dissolved waxes, variable mixtures of gummy resins, balsams, terpenes, and similar substances together with other complex constituents. They are storage, waste, and residual products of very variable chemical structure, and most of them were originally secreted by cells and glands of different kinds of plants. A study of solvents for the separation of these substances from peat materials with par-

ticular reference to the differences in the action of benzol, toluol, xylol, and phenol, as well as of ether and alcohol, is just beginning to be made (*42*).

Many of the extracted substances, such as, "fichtelite," "ozokerite," and others (*19*) do not belong to the true fats and oils. Fatty substances when breaking down yield a considerable series of fatty acids without the acid H-ion. They give off more heat on burning, because they contain a small proportion of oxygen but relatively more carbon and hydrogen than the carbohydrates. Waxes and resinous substances are especially abundant in the foliage of woody heaths and evergreen trees. Hence, peat layers derived from these plants carry more of the materials extracted by ether and other solvents than the peats derived from sedges, reeds, or mosses. Treatment of peat with phenol yields more extract than with any other solvent, and the yields are considerably increased by the use of higher temperatures (*43.*) The products, moreover, have considerably higher melting points than the other extraction products. The extracts resemble more or less the bitumin from lignites. Schneider and Schellenberg (*42*) found that the action of solvents depends also on the relative age of the peat material, the deeper and older layers in a given peat profile with the higher proportion of resistant material giving the higher yields. The evidence presented by them is, however, insufficient, and the error in the conclusion has been demonstrated by the work of Zailer and Wilk, and others cited in Table I of Bulletin 802 (*8*).

There are marked differences in the percentages of ether-alcohol extract obtained from the several air-dried peat materials of this country. In general, the extract figures shown in Table I vary from 0.15 per cent in the Lake Okeechobee, Fla., sedimentary peat to 3.78 per cent in the reed peat from Wood County, Wis., which underlies a conifer forest vegetation and shrub heaths as ground cover. The same range of variation in the percentages of this fraction is, furthermore, seen in Table II where the results are stated on an ash- and moisture-free basis. The finely divided sedimentary peat from Lake Okeechobee, Fla., contains a minimum of 0.26 per cent and the Wood County, Wis., reed peat yields the maximum of 4.38 per cent. Together with the figures in Table IV these results again indicate that the botanical composition of the plant remains forming peat layers is a considerably more important factor than the depth below the surface. In the sedge peats from Chelsea, Mich., as well as in the sphagnum peats from Fairbanks, Alaska, and from Calais, Me., the presence of relatively large amounts of ether-alcohol extract is probably due to an admixture in the peat samples of leached plant residue from heath shrubs. There is a tendency towards the attainment of a maximum value for the ether-alcohol fraction, which appears to be more or less closely correlated with the degree of decomposition of the peat material. The data in Tables I and II show that increasing accumulation of the more resistant organic material may cause a steady increase in the waxy, resinous residue. The analyses for the partly decomposed hypnum peat from Phillips, Wis., for the woody peats from Margie, Minn., and from Charlevoix, Mich., and for the sedge and sphagnum peats which have undergone a partial decay, agree in showing this tendency. Thus the percentages of ether-alcohol extract in the several peat materials depend not only upon the type of the plant remains, but also on the stage of alteration and upon the predominant admixture of distinctly heath components.

INORGANIC MATTER IN DIFFERENT PEAT LAYERS

Mineral matter is present in peat materials either incorporated in organic compounds, in cell walls, as crystals and incrustations, or held by the absorptive power of the finely divided organic matter. Foreign material constitutes not infrequently the larger portion of the ash content in certain classes of peat deposits. It is brought in during the accumulation of peat layers by wind and water as dust, silt, or clay, and through the growth of shell mollusks, diatoms, sponges, and other organisms. The inorganic contamination may be disregarded for the present, since it is not a part of plant ash or that of peat, although reported as such in peat analysis. In most investigations concerning the agricultural value of peat land, the ash and its "standard fertilizer" ingredients are the only factor considered in grading peat areas. However, the quantity and character of the mineral matter vary from point to point in the same deposit, and even in the same layer. Owing to the constant evaporation of water from the surface of cultivated peat areas, many of the salts accumulate at or just below the surface peat soil. The amount and constituents vary much, because the presence of mineral matter, such as

clusters of calcium sulphate crystals, nodular concretions of iron, marl, and surface concentrations of lime or alkali, are determined largely by the chemical nature of circulating ground waters, by overflow, or by the excess of soluble salts carried in the mineral subsoils. In order to guard against a wrong interpretation of field data, it is necessary to remember, therefore, that the chemical nature of the groundwater or of the geological formation of a region does not in itself determine the fertilizer needs of peat land in that region.

The ash of the peat materials, indicated in Table I, consists of the total mineral matter left when the organic matter is completely burned. The differences in ash content range from 1.07 per cent in the sphagnum peat from Calais, Me. to 65.48 per cent in the Fremont, Ind., gelatinous material. The figures for the three samples of bog moss peat, for the Charlevoix, Mich., heath shrub and sedge peat, and for the Wisconsin reed peat indicate that a considerable loss of mineral matter has taken place by leaching. The sedimentary types of peat, respectively from Fremont, Ind., Middle River, Calif., and Lake Okeechobee, Fla., show a relatively high ash content; this is to be correlated, undoubtedly, with the general siltation of the water basins from which these samples have been obtained. The ash content of the cultivated reed "muck" from Plymouth, Ohio, and of the drained and aerated woody forest peats from Charlevoix, Mich., and Winnamac, Ind., exhibits the influence of soil-forming processes; the increase during decomposition is due partly to the adsorptive power of the finely divided organic components, but mainly to the transformation of organic matter into carbon dioxide.

Interesting information on the ash constituents of plants and peat materials is reviewed by Hoering (*19*). The recent work of Hibbard (*18*) indicates that the ash of "tule" (*Scirpus lacustris* var. *occidentalis*) is similar to kainit as a source of potash, but that the leached plant remains in the California tule peat lands have lost most of the mineral material worth recovering. Miller (*31*) gives data upon the inorganic composition of sawgrass (*Cladium effusium*) from the Florida Everglades. But the evidence submitted by him regarding layers of peat supposedly resulting from the accumulation of the remains of sawgrass is palpably insufficient. The thorough and accurate studies made by Zailer and Wilk (*53*), Bersch (*2*), Birk (*4*), and Minssen (*32*) show the changes of the inorganic material in different kinds of plants forming peat and in the corresponding layers of peat derived from them. These authors give a large number of analyses; they also point out that the ash content does not increase with the depth of the deposit, but varies with the nature of the peat profile, and with the contamination by extraneous sand, silt, or lime. In general, the work conducted by these and other investigators shows that silica is undoubtedly one of the most stable mineral constituents when present in a peat layer, while potash and to a less extent, phosphorus leach readily from different types of peat. On the other hand, it has not been emphasized sufficiently that iron and sulphur compounds are among those which not infrequently affect extensive areas of otherwise desirable peat land and may cause root rots. The deposition of dissolved iron in plant remains and its gradual replacement of the organic matter has been shown in a former publication (*8, Pl. 1*). The injurious effect of the accumulation of soluble iron within the tissues of growing plants reported by Sherwin (*47*) further emphasizes the necessity of modifying the accepted fertilizer standard of ash analyses.

Continued submersion in water makes various mineral salts comparatively unstable in peat. The darker color of peat samples taken from greater depths below the surface, the production of sulphuretted hydrogen and of methane, the reduction of sulphates and possibly of phosphates (vivianite) together with those other changes which are characterized by the withdrawal of oxygen not only from the water but also from the carbonaceous and nitrogenous substances in the organic matter, indicate the nature of the reduction action (*7*). The absorptive power for certain mineral substances appears to be localized in finely divided peat. The relative "saturation" of its colloidal constituents probably influences to a considerable extent the character and the varying salinity of the peat soil solution, the kind and concentration of various bases, and the rate at which microorganisms and filamentous fungi decompose peat materials. The low mineral salt content of certain types of fibrous peat soils in all probability accounts for the lack of nutrition in the grasses of such peat-land pastures. Further work is greatly needed to determine the movement, absorption, and the losses and exchange of soluble mineral material in layers of

woody, fibrous, and sedimentary peat at different depths, as well as during the transformation of drained surface horizons to muck and humus.

CONCLUSIONS

Results obtained with 20 different kinds of peat indicate the feasibility of the application of foodstuff analyses for the determination of qualitative differences in sedimentary, fibrous, and woody peat materials. These data point to contrasts between peat materials in regard to their nitrogenous substances, lignin, cellulose, and other carbohydrates, waxes, resins, and similar plant products. Their value is necessarily limited, since it is only possible to utilize analyses of the same character as those used for crops and feeding stuffs. However, they illustrate the fact that a close connection exists between the botanical and the chemical composition of the main groups of peat. By the application of this method it becomes possible to correlate variations in the chief groups of organic compounds with structural differences in the profile of peat deposits, to follow the progress of decomposition in drained surface peat soils, and to determine the degree of chemical alteration taking place in the several layers of peat below the water level. The analyses show the wide differences in agricultural value of the several kinds of peat, on the basis of the ratio of nonnitrogenous to nitrogenous materials, and the limited usefulness of various kinds of raw peat as a source of food for livestock or for soil microorganisms.

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BOTRYTIS ROT OF THE GLOBE ARTICHOKE [1]

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INTRODUCTION

Gray mold rot causes heavy losses in practically all truck-crop shipments made during the cooler months of the year, especially from California. Cross-inoculation experiments have indicated that the strains of *Botrytis* sp. affecting the Globe artichoke (*Cynara scolymus* L.) are pathogenic to those vegetables subject to decay from the Botrytis usually called *B. cinerea* Pers.

In their survey of plant diseases as they occur on the market, Link and Gardner [2] reported gray mold rot (*Botrytis* spp.) as the only disease affecting the buds of the Globe artichoke in transit and on the market.

The disease has been observed in the United States wherever artichokes have been examined. Practically all the artichokes marketed in this country are grown in Contra Costa and San Mateo Counties, Calif., mostly on the rich loams and clay loams of the narrow strip along the seashore from Montara to Pescadero; some are grown also in the canyons extending from the seashore into the mountains. The buds are shipped in refrigerator, freight, and express cars to eastern markets, 50 per cent of the total shipments going in 1922 to New York.

The economic importance of the Botrytis rot of the artichoke is suggested by abstracts which the plant disease survey made of inspection certificates issued by the food products inspection service of the Bureau of Agricultural Economics. Although the inspection service does not examine all cars arriving terminal in markets, the abstracts of inspections made show the artichoke to be affected by only one rot, which is practically the only cause of loss in transit. Ninety-six per cent of the car-lot inspections made during 1918 to 1923 report losses of 2 to 100 per cent due to Botrytis, the remainder being due to freezing. About 10 per cent of the inspections show losses of 50 per cent and over; 20 per cent show loses of 20 per cent and over; 70 per cent show losses of less than 20 per cent. These losses are considerable since the market price of a car (470 to 480 boxes) is $1,800 to $2,500, depending upon market conditions, with a freight and icing charge of about $375 from California to Chicago. The presence of even slight decay necessitates expensive resorting and considerable loss to wholesalers and retailers. Severely affected buds are a total loss, although slightly affected ones can be sold at a reduced price.

Inquiries from receivers, carriers, and food products inspectors of the Bureau of Agricultural Economics in 1919 to 1922 have shown a prevalent supposition that the serious losses on artichoke shipments were mainly attributable to freezing injury and chilling. There were consequent mutual recriminations among growers, shippers, carriers, and receivers, each believing some other responsible for the occurrence of such injury. None had realized that the great losses in transit might be connected with the presence in the field of a certain fungus.

Reduction of such wastage and loss in transit, with fixing of the responsibility for these, calls for definite knowledge concerning the source and mode of contamination, the time, place, and manner of infection, and the development and spread of the disease. As there were no experimental data adequate for formulating control measures and placing responsibility for losses in vegetables from this source, the artichoke was chosen for investigation of the disease, on account of certain advantages which it affords for type

[1] Received for publication Feb. 25, 1924—issued January, 1925. Paper from the Cooperative Laboratory for the Investigation of Market and Transit Diseases of Vegetables and Fruits, the U. S. Department of Agriculture and the University of Chicago cooperating.

[2] Link, G. K. K., and Gardner, M. W. Market pathology and market diseases of vegetables. Phytopathology 9: 497–520. 1919.

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study, even though this product is less important than the majority of crops affected by the fungus.

The work discussed in the present paper leads to the conclusion that, without previous or attendant freezing injury, a species of Botrytis, to be placed in the *cinerea* group, can produce the condition responsible for the deterioration and consequent losses in transit.

DESCRIPTION OF THE DISEASE

The lesions which indicate the disease are so small and inconspicuous that they are often overlooked in harvesting and packing, the more so since they vary with external conditions.

In the field the lesions are usually but 1 to 2 mm. in diameter on actively growing buds of vigorous plants, and are generally restricted to the tips of the scales. They are sunken and brown to black. Sometimes buds severely affected by Botrytis are found on aging plants, or on vigorous plants after the close of the active period of bud production (October to May). These usually shrivel and wither, and are covered with sporulating mycelium.

Extensive lesions of the moist type can be seen on vigorous plants in the field. They usually start in the wounds made when the buds are cut, whence they progress down the flower stalks and spread to the lateral branches and even to the main stems of the plants.

The lesions may occur anywhere on the bud. Spore infection seems most frequent on the cut stem and at the tips of the scales which are generally somewhat split by growth tensions. Contact with the mycelium leads to infection of any part; under moist conditions the spider weblike growth of the mycelium starting from one original center of infection frequently involves the entire bud, the rot progressing from scale tip to scale tip and down into the scale so that finally the entire bud is destroyed (pl. 1, A, B). Infection at the base of the scales or in the stem may destroy the entire stem and receptacle so that the bud readily falls apart (pl. 1, B). Under dry conditions, an affected bud then becomes a shriveled mummy.

Under moist conditions the lesions on the buds have definite slightly water-soaked borders, semimoist to wet, odorless, reddish-brown to brown, and generally with abundant growth of sporulating mycelium on their older portions. Under drier conditions the advancing edge is not water-soaked, the affected tissues are dry and firm, brown to black, and show no aerial development.

THE CAUSAL ORGANISM

ITS ISOLATION AND IDENTITY

Isolations of *Botrytis* sp. made from the flower buds of artichokes collected on the Chicago market, and from the stalks, stems and buds collected in the fields near Half Moon Bay, Calif., furnished the cultures used in the present study. A lot shipped from the fields was of particular interest because the entire plants including flower buds were frozen solid during transit. Viable cultures apparently identical with strains collected on the market were isolated from this material. One strain isolated from the interior of a frozen stem showing a brown surface lesion has maintained characteristics seeming to indicate a difference from all other cultures. On first isolation plates this culture, No. 255, grew more like a Fusarium than a Botrytis; but after one week's growth sclerotia began to develop and conidia were observed around the edges of the plate. The cultures of this strain always produce a honey-yellow[3] color in the substratum and fine ivory-yellow mycelium on potato-dextrose agar plates, as contrasted with the smoke-gray to light grayish-olive mycelium and grayish-olive substratum of the other strains. The gray strains also make greater vegetative growth and sporulate much more freely and abundantly than the yellowish strain.

Morphologically, there do not seem to be great differences among the cultures under observation. The mycelium is pale smoke-colored at first,

[3] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

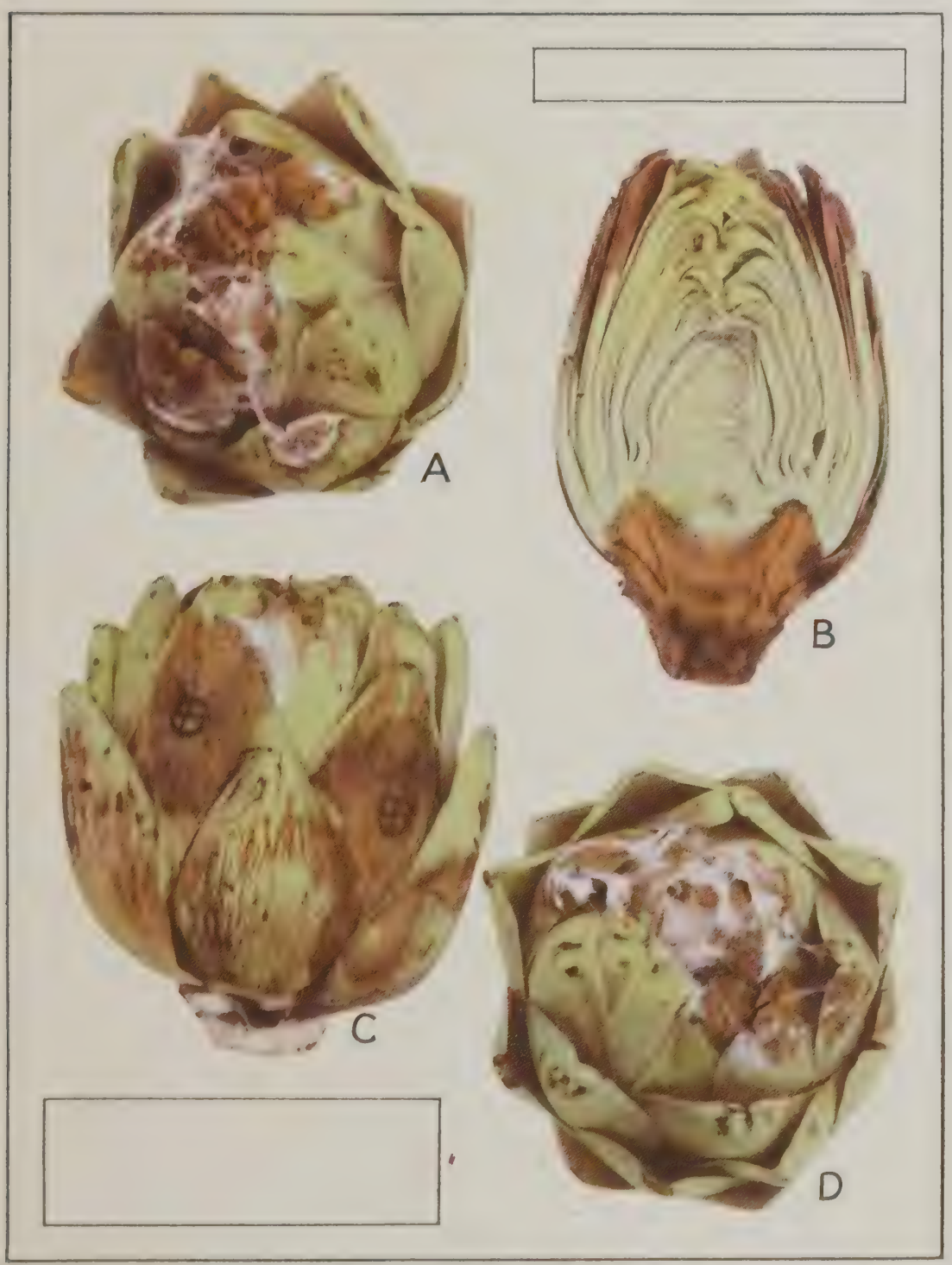
EXPLANATORY LEGEND FOR PLATE 1

A. Artichoke held in a humid atmosphere, showing lesions and spider weblike growth of Botrytis mycelium over the surface.

B.—Longitudinal section through bud showing stem and receptacle infection.

C.—Artichoke showing points of inoculation and lesions resulting therefrom. Mycelium was inoculated into wounds whose position is indicated by circles. Dark discolored areas show limits of fungus advance. The blotched discoloration on lower two scales is due to bruising.

D.—Lesions and mycelium on split and wounded tips of scales resulting from inoculation with a spore suspension.


A
B
C
D

becoming gray to grayish-brown with age. Conidia are produced in rather loose clusters on the rounded to slightly inflated ends of the tips and lateral branches of the upright sporophores. In old cultures sclerotia are present, which on germination give rise either to vegetative mycelium or to short, rather compact tufts of conidiophores with grayish-brown conidia.

With the possible exception of culture No. 255, whose spores are usually slightly smaller than those of the others, there is very little variation in size of the conidia of the various cultures. In the seven cultures used in most of the studies, the spores ranged from 8.2 to 10.5μ in width and from 11.3 to 14.4μ in length, averaging 9.4 by 13.1μ.

The growth characteristics and spore measurements of these fungi are well within the range and description of the fungus usually designated *B. cinerea* Pers. In view of the existing confusion regarding species within the genus Botrytis, it has been thought best to refer to the organism or organisms under consideration as the *B. cinerea* type, since, as stated in the beginning of this article, practically all Botrytis strains occurring on vegetables are identical and can be grouped as the *B. cinerea* type (with the possible exception of culture No. 255).

TEMPERATURE RELATIONS

In temperature experiments on plates and tubes of potato-dextrose agar, it was found that one culture, No. 1610, just isolated from an artichoke bud, was able to make radial growth ranging from 1 to 7 mm. within seven days at −2° C. None of the other strains which had been cultured on artificial media for some time were able to grow under the above conditions, although in other respects the strains appear very similar. This suggests that a difference in pathogenicity may be expected in cultures, due to loss of vigor in growing on artificial media. All other strains grew from 0.5 to 2 mm. in radius at −1.5° to −1° in seven days. During the seven-day period at 0° these strains grew 7 to 14 mm. in radius while the newly isolated strain No. 1610 under parallel conditions grew but slightly faster. At higher temperatures growth becomes more rapid until at 20° to 22° an average daily radial growth of 5 to 7 mm. is made. From experiments conducted at still higher temperatures it appears that the optimum for growth on potato-dextrose agar lies between 22° and 25°. These observations agree well with the results of Brooks and Cooley [4] for *B. cinerea*.

The reactions to temperatures above 31° are approximately alike for the various strains in plate cultures. Usually very little growth is made. An increase of 1 to 2 mm. observed in some cases was mostly a very fine aerial mycelium arising from the inoculum. A few sporophores bearing conidia were also noted developing from the inoculum of one strain. But their vitality is so reduced at the above temperature that the organisms are not likely to produce appreciable decay.

SPORE GERMINATION

The spores of all strains germinate readily in hanging drops of sterile water and on the surface of nutrient agar plates. The majority germinate within 24 hours at room temperature and above, while a smaller percentage form germ tubes at temperatures around 0° C. The extreme range is wide, as successful germination has been obtained at temperatures as low as −2° and as high as 32° C.

An experiment in which the temperatures ranged between −2° C. and −1.5° with a mean of −1.75° showed good germination in the three strains tested. In a similar experiment in which spore dilutions of four different strains were poured upon plates of potato dextrose agar and placed where the temperature varied between −1° and −3°, good germination was obtained within 48 hours. From 50 to 75 per cent of these spores germinated, and their germ tubes ranged from 20 to 90μ in length. It appears from these and other tests that mature conidia falling upon a suitable nutrient medium can germinate and make slow growth at temperatures below 0°. In sterile water at the above temperatures, however, germination seldom occurs, or is at most rather feeble compared with growth on a nutrient medium.

Spore dilutions on potato-dextrose agar plates held at 31° to 32° C. give 50 to 90 per cent germination within 24 hours. Growth of the germ tubes continues 4 or 5 days, then usually stops when they reach 100 to 200μ in length. This would seem to indicate a temperature of 32° as the maximum for growth from conidia in the strains under observation.

[4] Brooks, C., and Cooley, J. S. temperature relations of apple rot fungi. Jour. Agr. Research 8: 139–163, illus. 1917.

PROOF OF PATHOGENICITY

The pathogenicity of the organism was established by its constant association with and isolation from lesions and by numerous inoculation tests made with artichoke and other vegetables.

Vigorous fresh-appearing buds, free from blemishes, were used. They were prepared for the test by making a fresh cut of the stem; by thorough washing; by dipping into 1:1,000 mercuric chloride solution for about a minute followed by thorough rinsing in sterile water. Longer immersion in corrosive sublimate solution was found to cause discoloration of the tender scales. Preliminary experiments and the controls proved the method adequate to remove or kill any spores on the buds.

To duplicate the method of inoculation which seems to occur naturally, both spores and mixtures of spores and mycelium were used as inocula. The spores were suspended in water and the suspension either poured or sprayed on the buds. These methods, especially the latter, approached as nearly as possible to the manner of inoculation in the field. Mycelium inoculation was made by inserting agar inoculum, or infected scales which generally had some mycelium on their surfaces, between the scales of sound buds. This duplicated the mode of inoculation involved when the disease spreads in transit. After inoculation the buds were kept in moist chambers or covered battery jars.

Inoculation experiments with infected scales demonstrated that the fungus can produce decay throughout its temperature range. As the buds freeze at −2° C., no tests were made below this temperature. None of the cultures which had been grown on agar for some time produced appreciable decay at −2°, whereas strain No. 1610, recently isolated from artichokes, produced decay. Four scales of a bud inoculated with this strain became infected to the extent of 6 sq. cm. after a week. The presence of the fungus in the advancing edge of the lesions was proved by microscopic examination and plate cultures. At −1° all strains produced decay. The percentage of successful inoculations increases steadily up to the optimum temperature of 22° to 25° C. Failures were infrequent at this temperature but increased rapidly above 24°; occurred in about 85 per cent of the attempts at 26°; and lesions were obtained but rarely between 26° and 32°.

Although infection was more consistently and rapidly obtained through wounds when mycelium was used as inoculum, the experimental evidence shows that wounds are not a necessity. The inoculum was applied at the tips, the middle, and the bases of the scales, as well as at the cut surface of the stem, and consisted of mycelium taken from agar plates (Pl. 1, C). Similar results were obtained by using infected scales as inoculum. Seventeen tests at 0° C. gave 12 lesions on wounded buds and 2 on unwounded buds. Fifty-one tests at 7° gave 46 lesions on wounded buds and 43 on unwounded buds. Fifty-one tests at 22° gave 48 lesions on wounded buds, also 48 on unwounded buds. Eight tests at 30° gave five lesions on wounded buds, none on unwounded buds. It was difficult to keep the humidity high enough to produce infection at the higher temperatures. Unfortunately, no apparatus was available for making controlled humidity tests.

Except at very low temperatures, inoculation and infection by contact seem limited much more by moisture than by temperature or by the presence of wounds. With adequate moisture, infection took place and was quite independent of wounds, except at very low temperatures. A saturated or nearly saturated atmosphere induces very rank development of mycelium, which, however, as in plate cultures, seldom sporulates unless subjected to a change in humidity. It was necessary to wrap each bud in waxed paper for keeping in moist chambers in the laboratory, in high-temperature incubators, or in incubators cooled by mechanical means which freeze out the air moisture. In low-temperature incubators, cooled by melting ice, wrapping was not necessary.

Experiments with spore suspensions gave negative results except on wounded tissue. Moisture was again a limiting factor, but even more decisively. In early experiments no infection was obtained at any temperature with spore suspensions sprayed or poured on wounded or unwounded scales. Wrapping was entirely inadequate when spore suspensions were used, but the difficulty was finally solved by fogging the chambers containing the buds once every 24 hours during the first three or four days. This was done by spraying water into the chambers with an atomizer. It was a very unsatisfactory method because there was no way of determining just what degree of humidity is essential for spore inoculation; but it duplicated field conditions in that the air

was saturated and water was condensed on the buds at least once a day.

In inoculation tests with spore suspensions made with wounded and unwounded buds at temperatures ranging from −1° C. to 26°, negative results were obtained at −1°, 0°, 0.5°, and 1.5°. Infection resulted occasionally at 5.5°, but only in wounds. The number of infections obtained was greater at 7° than at 5.5°, and still greater at 10°, with a maximum between 15° and 21°. Between 26° and 32° infection was obtained only occasionally. All infection obtained started in artificial or natural wounds (Pl. 1 D). Scales which had been wounded and dipped in spore suspensions developed lesions of 2 to 5 sq. cm. with sporulating mycelium on the surface in nine days at 26°.

The presence or absence of wounds seems, therefore, the most important limiting factor so far as infection by spores is concerned, with moisture, and then temperature next in importance. For infection by mycelium, however, moisture is the main limiting factor, temperature and wounds ranking in the order named.

Infection with spores was not obtainable when the tissues were not wounded. Wounds are abundant and inevitable, however, on both the growing and the harvested buds. There is more or less tearing of the scale tips during growth, for they are curved inward and indented (Pl. 1, A, D) so that tensions arise at the spine and lead to tearing as the scales enlarge. A large wound is made when the bud is cut from the plant in harvesting (Pl. 1, B), and later when buds are crowded into crates for a tight pack there is more tearing of the scales and considerable bruising of the sides as well.

Holding tests made at 7° C. show that the buds of a lot which appear sound at the beginning of a test develop Botrytis decay progressively, and eventually almost all show decay. Possibly this is due to a weakening of the tissues. It is a general observation in the field that *Botrytis* readily attacks aging or weakened parts of plants, such as the outer dying leaves of lettuce or celery. Field observations indicate that the age or vigor of artichoke buds is a factor, inasmuch as the small buds produced at the close of the seasonal productive period or by old plants show greater decay than buds from more vigorous plants, some inspection certificates reporting 4 to 10 per cent decay in large buds and 20 to 30 per cent in small ones. It has also been observed that tissues killed or weakened by overheating or freezing are more readily attacked than healthy ones.

THE DISEASE

PLACE OF ORIGIN, SPREAD, AND DEVELOPMENT

Observations in packing houses indicate that even though some buds with small lesions are packed, by far the greatest number of boxes contain few buds showing any signs of lesions or of the fungus. Field observations indicate that spore contamination in the field is probably the original and chief source of the disease in transit. Here can be found buds and other parts of the plants covered with sporulating mycelium. A much more serious source of infection, however, is the immense amount of plant trash allowed to lie in or adjacent to the fields, which is often covered with an extensive sporulating growth of Botrytis that liberates clouds of spores whenever disturbed. The great quantities of plant trash exist because the bearing plants, which resemble huge thistles, are cut down annually in May and their roots allowed to sprout or sucker. The new tops come into bearing during September and October, and in the fourth or fifth year, when buds begin to be small and hard, the plants are replaced by new shoots.

Examination upon arrival of buds taken out of boxes not previously opened shows that the lesions usually start at the tips of the scales or at the cut stem surface. This indicates that the original infections come from spores that lodge in these places and that secondary infections in transit are primarily by contact. Secondary infection by spores also may take place, since infected scales frequently are covered with sporulating mycelium.

That the bud surfaces carry either spores or mycelium of Botrytis is easily demonstrated by taking buds from packing boxes and exposing them to favorable temperature and moisture conditions. Botrytis rot develops almost invariably. In fact, if the buds were not washed and sterilized, it seemed impossible to have control material come through the experiments, which lasted from several days to several weeks, without developing Botrytis rot. That the contaminations were mostly on the surface is proved by the fact that a few minutes' dipping in corrosive sublimate solution sufficed for sterilization. Thorough washing was also frequently sufficient. Yet occasional development of decay in buds so treated proved that incipient but invisible infections were also present.

It was found that spores or incipient infections may lie dormant for considerable periods if conditions are unfavor-

able, and then begin or resume growth when conditions become favorable. That transit gives ample time for the development of lesions already present and the starting of new ones is indicated by the following data: At 7° C. lesions with a radius of 0.5 to 2 mm. developed in 7 days when wounds were inoculated. Scales inoculated with spore suspensions at 25° developed lesions which had an area of 2 to 5 sq. mm. on the ninth day and were covered with sporulating mycelium. Lesions increased in radius at 10° at the rate of 1.5 mm. every 24 hours; at 15 to 20°, at the rate of 4 to 5 mm.; at 27°, at the rate of only 2 mm.; and at 30° there was no increase. The maintenance of an average temperature of 7° is very good for a refrigerator car. Thus temperature conditions in transit are not unfavorable for the development and spread of the decay.

RELATION TO TEMPERATURE IN THE FIELD AND IN TRANSIT

The optimum temperature for growth of Botrytis lies between 22° and 25° C. The range from this to the maximum of 32° is only 7°, whereas the range to the minimum extends beyond −2°, or more than 27°. Unlike many other fungi, this fungus can make a very appreciable growth and sporulate between 5° and 10°.

The temperatures of the leading artichoke-producing section in California are not unfavorable for the growth of the fungus. The nearest weather reporting station where weather is comparable to that of the Half Moon Bay section is San Francisco, Calif. Here the temperature rarely exceeds the maximum for *Botrytis cinerea* Pers.; the lowest temperature is above its minimum and the mean is below rather than above its optimum.[5] At the annual mean temperature of the region (54.9 °F.), the fungus can enlarge lesions on inoculated buds at the rate of about 4 to 5 mm. per day under favorable moisture conditions.

Abstracts of food products inspection certificates show that the amount and severity of the decay of artichoke buds in transit vary with the temperature. Almost always the decay is heaviest in the two upper layers of car-lot shipments, and in these it is most severe at or near the doors. The following are typical reports: "Ten per cent decay in fourth and fifth layers; none in lower three"; or "Decay in two top layers, 40 to 60 per cent; in lower two, 2 per cent," or "Practically no decay in boxes near bunkers; 30 to 50 per cent in boxes at doors" and "Two top layers, all buds decayed; two bottom layers, slight decay." The direct temperature relation is shown by the temperature report for the last above-mentioned car, in which the temperature of the artichokes was 21° C. at the top of the load in the door, 11° at the bunker, and 4.5° at the bottom of the load at the bunkers.

RELATION TO MOISTURE IN THE FIELD AND IN TRANSIT

The experiments show that while moisture ranks second to wounds as a limiting factor for infection by Botrytis spores, it ranks first as a limiting factor for mycelial growth and infection by mycelium. For sporulation, however, changes in humidity are more important than continuous humidity.

Botrytis rot is generally reported as serious in sections having abundance of foggy weather or in other sections during foggy seasons. Since few regions offer such consistently favorable conditions as the Pacific coast, this probably accounts for the prevalence of Botrytis rot in vegetable shipments from California. The artichoke sections of California lie within the coastal belt of California where fogs are a daily occurrence, so characteristic that the cooperative growers association markets its best brand of artichokes under the trade name "Fog-Kist." During the growing season fogs drift in from the sea every afternoon and are not dissipated until late the next morning, so that vegetation is thoroughly wetted at least once daily.[6]

The artichoke buds are packed in paper-lined boxes which are generally loaded into iced cars. In the early part of the season buds are packed in iced drums. It was found that condensation of water frequently takes place in the buds when the boxes are first placed in the iced cars and that often the buds are moist when they are unloaded in receiving markets. Therefore moisture conditions in transit are also favorable for mycelium development, sporulation, infection, and development of lesions.

[5] U. S. Dept. of Agriculture, Weather Bureau. SUMMARY OF THE CLIMATOLOGICAL DATA FOR THE UNITED STATES, BY SECTIONS. REPRINT OF SECTION 14, CENTRAL AND SOUTHERN CALIFORNIA. 25 p., ILLUS. 1912.

[6] PALMER, A. H. FOG ALONG THE CALIFORNIA COAST. Mo. Weather Rev. 45: 496–499, illus. 1917.

CONTROL OF THE DISEASE

Two lines of control suggest themselves. The first and most effective, if it were possible, is prevention of contamination and, to a lesser extent, of primary infection in the field; the second is prevention of primary spore infection and of secondary contamination and infection in transit.

There was no opportunity for trial of protective sprays or dusts. In general, these have not been very successful in combating diseases caused by *Botrytis* sp. The greatest promise of control in the field lies in sanitary measures, such as the destruction of plant trash. Yet, even though scrupulous sanitation in the artichoke fields would very much lessen the amount of contamination, it would not necessarily eliminate Botrytis entirely, since coastal California weather conditions allow the fungus to grow saprophytically and parasitically on a great range of wild and cultivated plants. Maintenance of the low temperatures which can be obtained in the best refrigerator cars can not completely control the disease because even though low temperatures above the freezing point of the buds retard infection, development, and spread of the disease, they do not control it completely, since the fungus can grow at temperatures low enough to injure and freeze the buds. Moreover, low humidity controls primary spore infection as well as secondary contamination and infection much more than do low temperatures. Infection by spores or by mycelium was not obtainable at any temperatures unless the buds were kept in a humid atmosphere. Unfortunately, equipment was not at hand to determine just what humidity is necessary for spore germination and for infection by spores and mycelium. Control of humidity in refrigerator cars at present is difficult, because the present method of cooling cars by melting ice inevitably leads to a humid atmosphere.

Since these experiments show that even though spore germination and growth can take place at temperatures between −2° C. and 5°, and since infection by spores (the primary method of infection) does not take place at temperatures below 5°, much infection might be prevented if it were possible to get the buds cooled to this temperature immediately after cutting. Once infection has taken place, growth of the fungus and the infection by mycelium, for which wounds are not essential, can take place at temperatures ranging from −2° to 32°. Commercially significant lesions can develop in infected buds at 0° during the 10 to 15 days necessary to get the buds to eastern markets. Nevertheless, maintenance of a temperature of about 5° C. is desirable, as preventing secondary infection by spores, retarding growth of the fungus, and lessening secondary infection by mycelium.

Though some tearing of the tips of scales takes place during growth and a large wound must be made when the buds are cut from the plants, more careful handling of the buds during harvesting and packing could eliminate a great deal of wounding.

Control of the disease is therefore a complex problem, and responsibility for losses in transit or storage is divided among all those who handle the crop. Growers should keep fields and their environs free from plant trash. The packer should avoid packing buds which show discolorations and lesions, since there are indications that buds cut in badly infested fields are more heavily contaminated than those from clean fields. He should not mix badly contaminated and clean buds, should handle them with the greatest care to avoid wounding, and should pack without delay and cool them at once to about 5°. The carrier should provide the most rapid car movement possible, because each additional hour increases the chances of infection with the consequent danger of larger and more numerous lesions. He should also provide a temperature of about 5° while the buds are in transit, and try to prevent saturation of the atmosphere in the car. It is of advantage to the dealer to receive this stock quickly. He should then keep it in fairly dry, refrigerated places if it must be held any length of time.

SUMMARY

1. A rot induced by a Botrytis of the cinerea type causes serious transit losses of shipments of Globe artichoke buds from California. Morphologically, the Botrytis strains isolated from the artichoke (except culture No. 255 from the interior of a frozen stem) seem to be identical with those generally obtained from other vegetables. Cross inoculation tests lend additional weight to the view that a Botrytis of the cinerea type is responsible for practically all Botrytis rot of vegetables.

2. Although disease is of practically no importance in the artichoke fields so far as quality and quantity of marketable buds are concerned, the fields are the original source of con-

tamination, where the fungus attacks vigorous growing plants to some extent, occurring much more frequently on aging plants and on plant trash.

3. The fungus has a wide temperature range, its minimum lying below —2° C., its optimum between 22° and 25° C, and its maximum at about 30° to 33° C. Infection was obtained experimentally throughout the temperature range of the fungus. It is obtained more readily below the optimum than above it. The rate of development of lesions is greater below the optimum than above it.

4. The temperature reactions of the fungus are essentially the same on both agar and bud scales. Mycelium can cause infection without wounds at all temperatures within its temperature range, whereas wounds are necessary for infection by spores.

5. Moisture is the principal limiting factor for both spore and mycelium infection. High humidity followed by a decrease in humidity leads to sporulation. Temperature and especially moisture conditions in the artichoke-producing section of California are favorable for the growth of Botrytis with consequent contamination of the buds.

6. Control involves field sanitation and possibly protective spraying or dusting of the buds; also careful handling to avoid unnecessary wounds in harvesting and packing.

7. Control also involves the maintenance of low humidity and of temperatures of about 5° C. in transit, although even at this temperature complete control is not possible after infection has occurred, because the decay, when once started, progresses at —2°, the freezing temperature of the buds.

8. Responsibility for losses in transit or in storage is not single, but generally rests severally upon growers, packers, shippers, carriers, and storage men, each of whom could contribute some measure of prevention.

THE DEPTH DISTRIBUTION OF THE ROOT-KNOT NEMATODE, HETERODERA RADICICOLA, IN FLORIDA SOILS[1]

By G. H. GODFREY[2]

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INTRODUCTION

In any measure taken to decrease the number of root-knot nematodes in the soil, one of the factors that must be taken into consideration is the depth at which they occur. Whatever treatment may be given, whether chemical, heat, or cultural, must be applied with the idea of reaching the large majority of the parasites, or its effectiveness will be greatly diminished. The experiments described in this paper were designed to give a rough determination of the relative root-knot nematode content of certain soils at different depths, and at different seasons.

LITERATURE

Only the most general information on this subject is available in the nematode literature. Cobb [3] indicates, in regard to soil-inhabiting nematodes in general, that they are likely to be most abundant near the surface and gradually decrease in numbers as the depth increases. Atkinson [4] found root knots on parsnips and tomatoes 15 to 18 inches below the surface of the ground. Larvæ released from such knots would naturally increase very greatly the numbers of nematodes at that depth, for a time at least. Bessey [5] stated, in connection with the overwintering of the organism, that the nematodes probably descend into the lower levels of the soil to avoid the cold. No exact information on this point appears to be available. Frank [6] mentioned the occurrence of galls on deep rooted plants, and discussed the possibility of there being a positive geotaxis due to gravitation.

METHODS

The general methods followed in this experimental work were to collect soil samples at the desired depths from thoroughly infested soils and to grow plants susceptible to root-knot in them under uniform conditions. The number of distinct knots that appeared on the roots within a given period was taken as an index of the number of nemas in the soil, the results being naturally purely relative. It was assumed that a sample producing twice as many knots as another sample had relatively twice as many nemas capable of producing infection. This assumption need not be exactly correct in order that the general results be comparable.

The soil samples were taken in successive layers at depths of 1 to 2 inches, 3 to 4 inches, 5 to 6 inches, 7 to 8 inches, 9 to 10 inches, 11 to 14 inches, 15 to 18 inches, 19 to 22 inches, 23 to 26 inches, 27 to 30 inches, and 31 to 34 inches, respectively. The samples were taken in quadruplicate, each sample being sufficient to fill a 6-inch flower pot.

In collecting the samples every precaution was taken to avoid contaminating any lot with soil from another level. A V-shaped trench was dug to a depth of 34 inches. This is indicated diagrammatically in Figure 1. With a large, flat trowel the 1- to 2-inch sample was taken from the top of the soil at one end of the trench, and the excess soil to that depth lifted and discarded. After taking the sample, the trowel was sterilized by dipping in a boiler of hot water. Then the next layer was removed, the trowel sterilized, and so on until the 11 sets of samples were taken.

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[1] Received for publication May 5, 1924—issued January, 1925.

[2] The writer acknowledges credit due to Dr. G. M. Armstrong, now of the Missouri Botanical Garden, St. Louis, Mo., for an active part in this and other nematode problems of the South during the summers of 1920, 1921, and 1922.

[3] COBB, N. A.—NEMATODES AND THEIR RELATIONSHIPS. U. S. Dept. Agr. Yearbook 1914: 457-490, illus. 1915.

[4] ATKINSON, G. F.—NEMATODE ROOT-GALLS. Ala. Agr. Exp. Sta. Bul. 9, 54 p., illus. 1889.

[5] BESSEY, E. A.—ROOT-KNOT AND ITS CONTROL. U. S. Dept. Agr. Bur. Plant Indus. Bul. 217: 36 illus. 1911.

[6] FRANK, B. UEBER DAS WURZELÄLCHEN UND DIE DURCH DASSELBE VERURSACHTEN BESCHÄDIGUNGEN DER PFLANZEN. Landw. Jahrb. 14: 164, illus. 1885.

For the first series of experiments the samples were placed in cloth bags covered with a double wrapping of heavy waxed paper. These were packed in canvas bags, the four samples from each depth together, and the entire lot packed in crates and shipped to Washington. The soil was then placed in 6-inch pots under good growing conditions in the greenhouse, and set to tomato seedlings, three to a pot. In about six weeks, when the earliest infections had reached maturity, and before the production of any secondary infections, the plants were removed, the roots washed, and readings taken on number of root knots per pot.

In the second series of experiments the soil samples were collected direct from the field into 6-inch pots, and the tomato plants grown in them in the propagation houses at the Plant Introduction Garden at Brooksville, Fla.

As stated above, the measure of the relative nematode content of the various soil samples was the number of knots that developed on the roots of the tomato plants grown in them. At first actual counts were made. Based on these counts, a scale was adopted to indicate the relative numbers as follows: 1 to 100 knots, *few;* 100 to 200, *medium;* 200 to 400, *numerous;* 400 to 600, *very numerous;* above 600, *extreme*. The experiments conducted the second year were based not on actual counts but on estimates, recorded in the terms just cited. These estimates were made by Robert Smith, who, because of his long contact with the nematode experimental work, had a very good conception of relative degrees of infestation. It was found by a few counts that such estimates were sufficiently accurate for the purpose.

Unfortunately, some features of these experiments that should ordinarily have received more attention had to be neglected, owing to the pressure of work elsewhere.

RESULTS

In the first series of experiments, made with an extremely sandy loam soil at Brooksville, Fla., collections and plantings were made at intervals of a little more than a month, a total of 10 being conducted from June 28, 1920, to May 27, 1921. The soil samples throughout were shipped to Washington, D. C., and plantings of tomatoes made in the greenhouse. At approximately six weeks after planting, in each case, the plants were lifted, the roots carefully washed, and notes taken on the relative abundance of root-knot infestations.

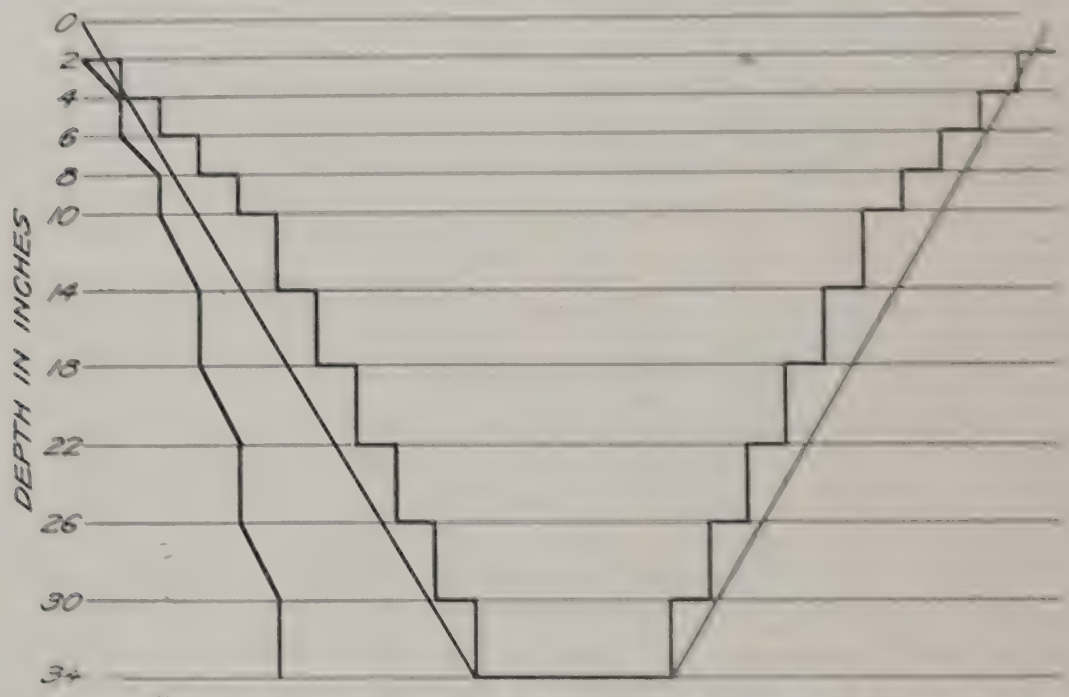

FIG. 1.—A diagram representing the trench dug for collecting soil samples at different depths

The chart (fig. 2) shows graphically, the relative depth distribution of the root-knot nematodes at different dates. In examining this chart it should be borne in mind that differences in infestation may not be significant as seasonal variations. More probably any great difference between one month and the one preceding or following it, for example, is due to distinct differences

in nematode content of the particular spots of ground where the samples were taken. For this series of experiments, these spots were unfortunately in widely different parts of the field, which naturally introduces an uncontrolled difference factor, even though the spots were chosen in each case on account of severe infestation in the crop preceding.

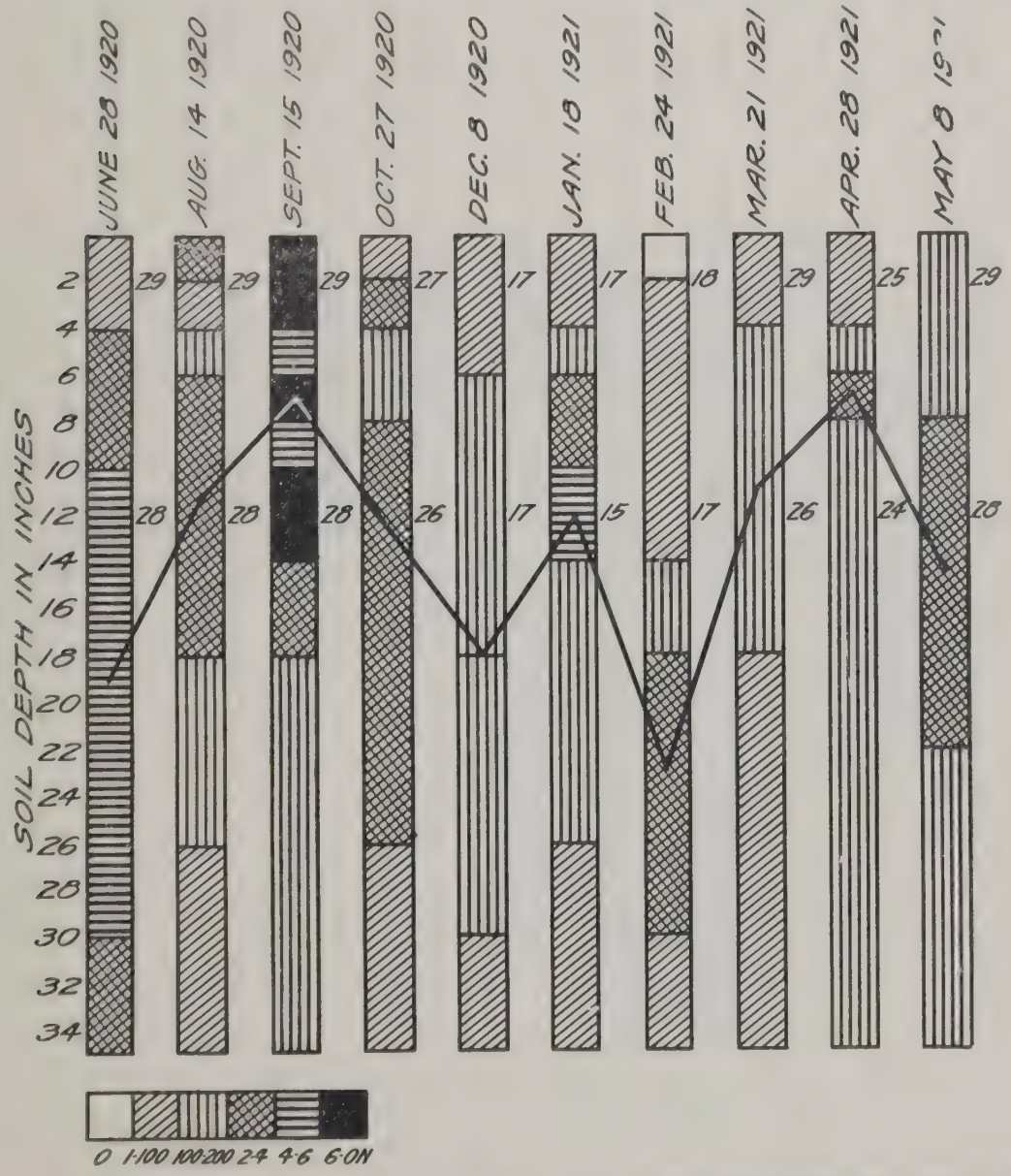

FIG. 2.—Depth distribution of root-knot nematodes in sandy loam soil at Brooksville, Fla., from June 28, 1920, to May 28, 1921. The shading indicates the approximate number of knots that developed on tomato plants grown in the soil samples. The results are the average of four samples. The curve connects central points of regions of maximum infestation. Temperatures in degrees centigrade at 2 and 12 inch depths are also given, these figures being the average for the week preceding date of collection of the sample, at the Plant Introduction Station at Brooksville, Fla.

The differences in nematode content between the different depths in the same spot in the field are within a reasonable degree reliable. The regions of maximum infestation, in the different spots, as determined by samples taken at different times, therefore, are roughly comparable. These regions were, on the whole, several inches beneath the surface. In June the nemas were most abundant below the tenth inch, and were numerous to the maximum depth examined, 34 inches. In August they were numerous down to 18 inches, and were present in medium or less quantities to the full depth. In September they were extremely abundant in the first 14 inches of soil depth. From December to April the nemas appear to have been less numerous throughout the soil than in the summer months, as might very well be expected. During that period the temperature was below that shown by experiment to be favorable for infection and development, and probably a large proportion of the many free larvæ and eggs may have succumbed to the many unfavorable conditions to which in all probability

they were subjected. Little is known of the actual bionomics of this nematode in its free state within the soil, of its movements and responses to various stimuli, of its relations to other forms of soil life, etc.

The temperatures, recorded on the chart for the 2- and 12-inch depths, show a considerable drop between October and December, but in all probability not enough to have much effect on the vitality of the organisms. The minimum temperature for the winter months at the 2-inch depth, 8° C., occurred on January 16 and again on February 26, 1921. The lowest weekly average at this depth was 17°, and the lowest weekly average at 12 inches was 15°. The curve drawn in the chart between the centers of regions of maximum infestation should indicate any marked seasonal movement, if such occurred. While there appears to be a slight depression in the curve during the winter months, it is probably not sufficiently great to be significant. If similar nematode distribution data and temperature records were secured in a region farther North, where freezing temperatures in the upper layers of soil occur, it is possible that they would show a significant seasonal movement of the nematodes in the soil.

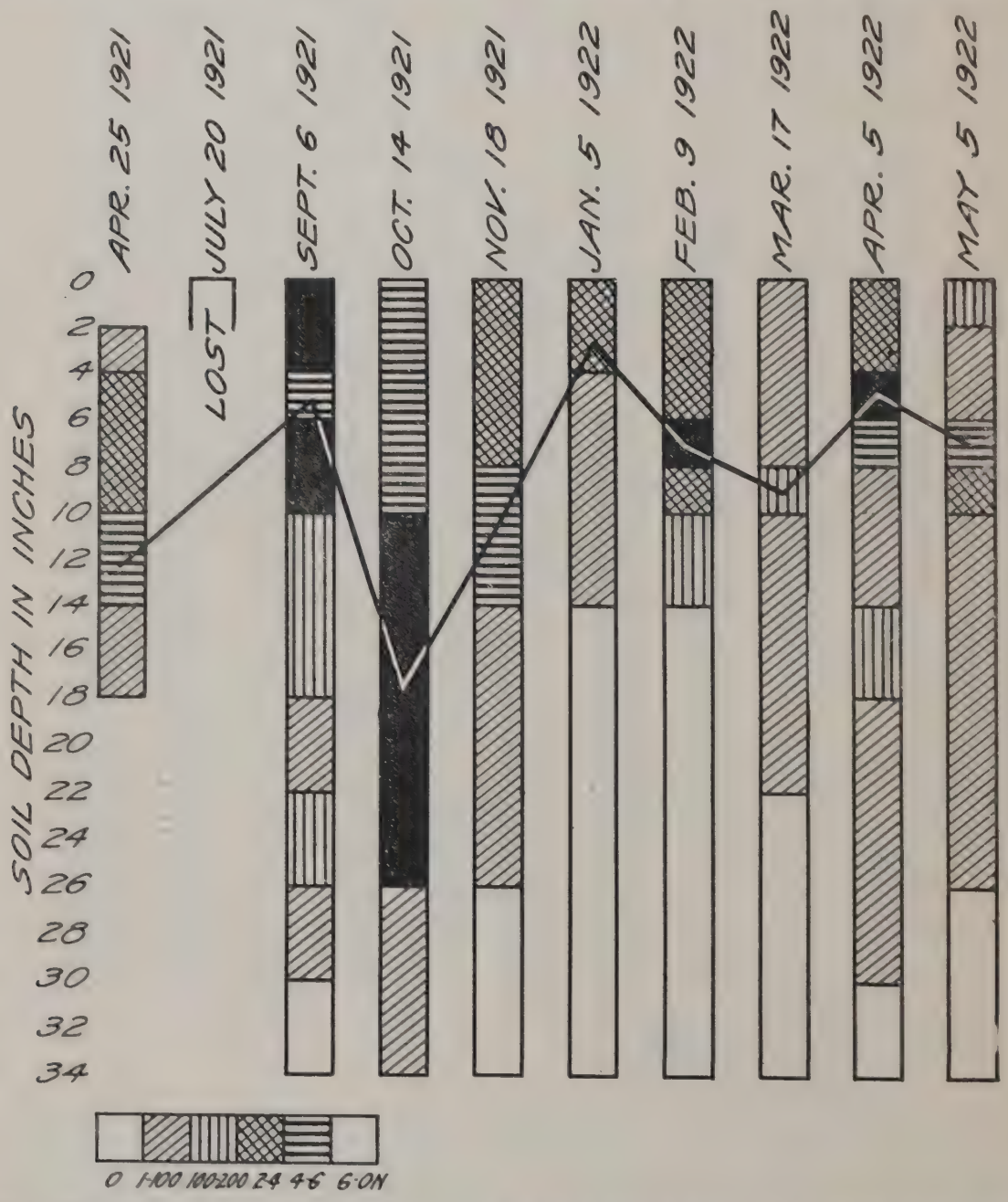

FIG. 3.—Depth distribution of root-knot nematodes in sandy loam or so-called "hammock land," with a heavy clay subsoil, on United States Plant Introduction Garden at Brooksville, Fla., June 28, 1920, to May 28, 1921

Figure 3 gives the experimental data on nematode infestation at various depths on hammock land at the Plant

Introduction Garden at Brooksville, Fla. Here the upper soil is a fertile sandy loam and the subsoil a heavy sticky clay. The subsoil very obviously limits the spread of the nematodes downward. Here, again, extreme variation from one month to another is shown, due no doubt to the actual difference in nematode content of the different areas sampled.

soil, considerable infestation occurs to the maximum depth sampled. The regions of maximum infestation are on the whole, near the surface, though below the ordinary plow depth. In some of the samples, notably those of October, 1921, and March, 1922, very abundant infestation was present deep in the soil.

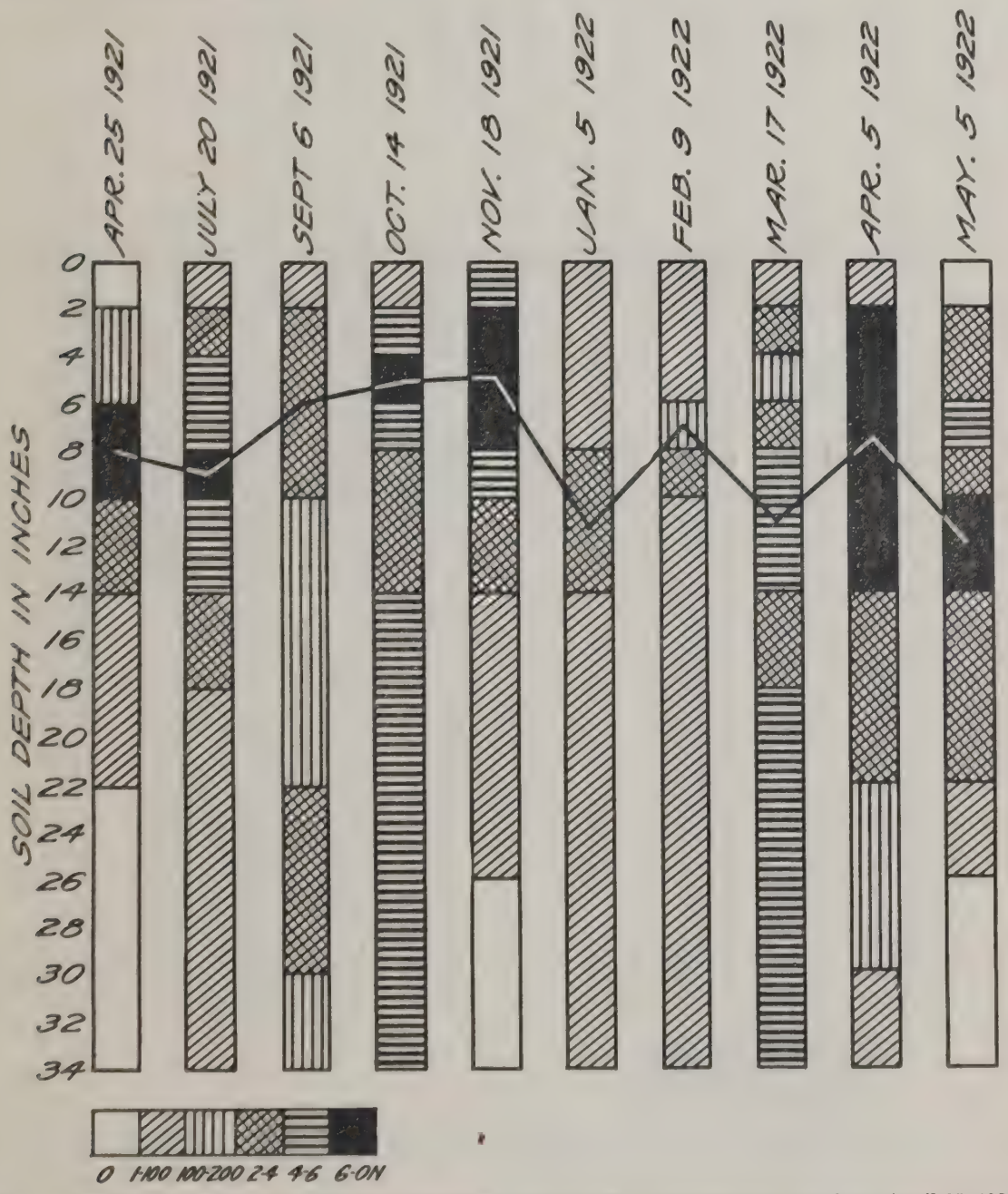

FIG. 4.—Depth distribution of root-knot nematodes in sandy soil at Brooksville, Fla., from April 25, 1921, to May 5, 1922

Figure 4 shows the results of an experiment in which sandy soil from various depths in a root-knot infested field at Brooksville, Fla., was sampled and potted, and planted with tomatoes, at the Plant Introduction Garden. The results in root-knot infestation were recorded by estimates of the relative numbers of knots present on the roots. As in the first year's samples of sandy

Experiment No. 4 was conducted in a similar manner to and simultaneously with experiment No. 3, except that Brooksville hammock land soil was used. The monthly variations in depth of the regions of maximum infestation, as shown in Figure 5, are certainly not significant, as regards seasons, and are obviously due to variations in the soils sampled. This is indicated by com-

paring this chart with Figure 3 in which depths of maximum infestation are distinctly different for the same months.

SUMMARY

Root-knot nematodes are often very numerous to a depth greater than that ordinarily reached by a plow. A considerable variation from one spot to another and from one month to another occurs in the depths of the regions of maximum infestation. Many factors no doubt enter into this, such as (1) differences in depth penetration of infested roots; (2) the relationship of time of taking soil samples to a period of heavy rainfall; (3) the height of the water table; (4) the type of the soil and the subsoil. The influence of such factors may be so great as to overshadow any seeming seasonal change, or at least any that may occur between one month and the next. In winter months a considerable reduction in total root-knot nematode content of the soil appears to be evident.

It is possible that regular seasonal movement does not occur to any great extent unless temperatures approaching freezing occur in the upper layers of soil. The experiments described here indicate that, in order to arrive at conclusive results in this regard, a large number of samples taken from different nematode-infested areas would have to be tested.

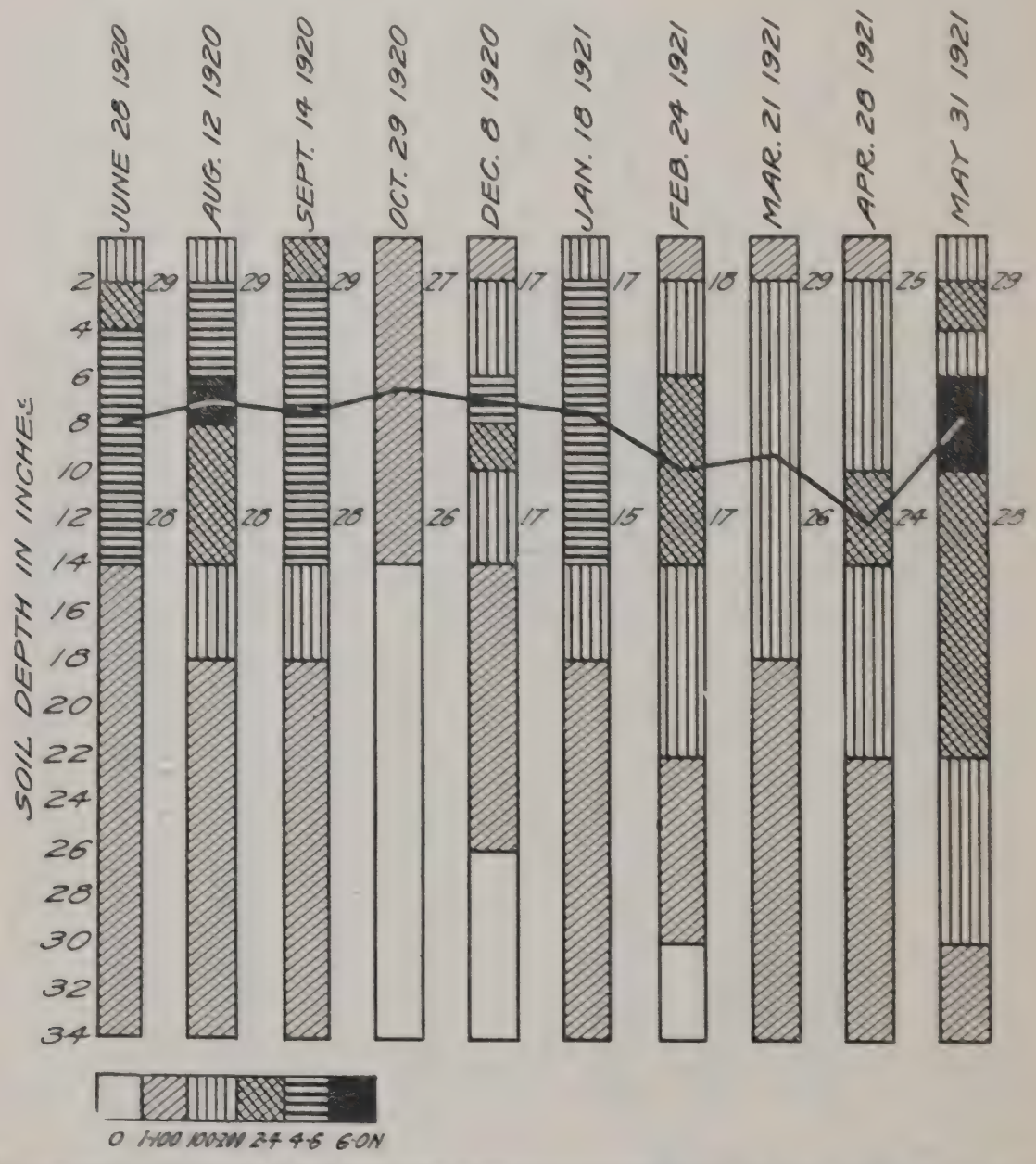

FIG. 5.—Depth distribution of root-knot nematodes on "hammock land" soil at the United States Plant Introduction Garden at Brooksville, Fla., from April 25, 1921, to May 5, 1922

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FREEZING INJURY OF APPLES [1]

By H. C. DIEHL, *Junior Physiologist*, and R. C. WRIGHT, *Physiologist, Office of Horticultural Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The growing and marketing of apples are foremost among fruit industries of the United States, as shown by the tonnage of fruit raised, value of the crop, carlot shipments, and quantities exported. Most of the apples are moved in the autumn, with the peak of the movement in October and November, as shown by data for 1918 to 1923 compiled by the Bureau of Agricultural Economics, United States Department of Agriculture.[2] However, considerable quantities are shipped in the colder months, and are thus liable to be exposed to low temperatures in transit. Consequently, very considerable losses are suffered by the apple industry every year from freezing in transit.[3] To this may be added the injury in cold and common storage houses when the temperature is allowed to fall below the freezing point of the fruit and to remain so for some time; also the injury from freezing when the fruit is still on the tree, which causes heavy losses in some districts. Detailed figures on the latter two sources of loss are as yet unavailable.

In spite of the importance of the apple industry, and the serious losses rendering protection for the fruit a matter of imperative necessity, the literature of the freezing problem as related to fruits has but few references to the apple.

Müller-Thurgau (*7*)[4] and Molisch (*6*) both made the observation that ripe apples may be exposed to a somewhat lower temperature without injury when thawed slowly than when the defrosting proceeds rapidly, but Chandler (*1*) was not able to demonstrate this for unripe apples.

Greene (*3*) reported experiments to determine the effect of freezing upon the keeping qualities of apples in cold storage, and to fix the temperature below which injury to apples could be expected to occur. He concluded that apples frozen on the tree could be stored safely provided the freezing was not too severe, that favorable weather followed it, and that thawing of the fruit had taken place gradually on the tree; also that apples frozen in cold storage at 24° F. or higher would show no injury if thawing took place gradually from 29° to 31°.

The investigations reported in the present article deal with determinations of the freezing points of most of our important commercially grown varieties of apples, with a study of undercooling and its relation to injury, and a determination of the effect of freezing on such matters as bruising of the fruit, visual injury, and keeping quality in storage, together with a discussion of the rate of cooling down and of freezing of apples in different types of packages under constant freezing conditions.

[1] Received for publication March 11, 1924—issued January, 1925.

[2] Carlot shipments of apples in the United States for the months September to February of the years 1918 to 1923, inclusive:[a]

Year	September	October	November	December	January	February
1918–19	8,070	26,680	13,563	6,320	4,044	3,679
1919–20	12,259	32,666	15,854	5,301	4,393	4,419
1920–21	11,043	37,284	23,087	8,875	6,046	6,698
1921–22	13,146	35,117	14,464	5,991	4,189	4,683
1922–23	14,787	32,052	19,512	8,229	8,438	6,257

[a] Data compiled by the Bureau of Agricultural Economics, U. S. Department of Agriculture.

[3] ROSE, D. H. DISEASES OF APPLES ON THE MARKET. U. S. Dept. Agr. Bul. 1253.

[4] Reference is made by number (italic) to "Literature cited," p. 127.

The apples used in these experiments were grown in various parts of the country, including the Pacific Northwest, California, New York, and the Maryland-Virginia apple district. A considerable number came from the orchards of the experimental farm at Arlington, Va. The varieties studied have been chosen primarily with reference to their commercial importance and are as follows: Yellow Newtown, Ben Davis, Grimes Golden, Delicious, Winesap, Rome Beauty, Esopus Spitzenburg, Jonathan, Wagener, York Imperial, Rhode Island Greening.

The methods followed in reading temperatures were similar to those employed in other fruit and vegetable freezing investigations (*4*, *10*, *11*). Temperature readings were obtained by means of single-junction copper-constantan thermocouples, of the type described by Taylor (*9*), in connection with a potentiometer and a galvanometer.

Some apples were frozen in metal cylinders immersed in an ice-salt bath, the fruit resting on cotton supported by a rack. The temperature of the air surrounding the fruit was read by a thermocouple suspended above and beside the fruit. Other apples were laid on dry boards in a freezing room held at constant temperature and frozen, the thermocouples being inserted in the usual way. A 10-junction thermocouple hung beside the apples exposed in the room. In all cases, however, the whole apple was subjected to the low temperature, the thermocouple being so inserted that its tip was located near the center of the fruit without being in the seed cavities.

Inoculation of the apples was effected by rapidly thrusting in and out of the fruit four or five times the thermocouple which had already been inserted in order to read the internal temperature. The term "inoculation" is used in this connection to mean any disturbance which will start crystallization of water, or ice formation, in the fruit. This caused a disturbance in the undercooled water films surrounding the thermocouple and brought on ice formation. It was found to be a more satisfactory method than tapping or bumping the fruit, since apples were sometimes inoculated with difficulty, even while considerably undercooled, and there was danger of serious bruising if they were inoculated by the latter means.

After freezing, the apples were removed from the cold room or freezing bath and placed in storage or subjected to the various treatments described later.

EXPERIMENTS AND RESULTS

FREEZING POINTS OF DIFFERENT VARIETIES OF APPLES

A considerable number of freezing-point determinations, made on different varieties of apples, has already been published (*12*).

TABLE I.—*Average and extreme freezing points of apples*

Varieties	Temperatures		
	Average	Minimum	Maximum
EASTERN GROWN	° *F.*	° *F.*	° *F.*
Baldwin	29.0	28.8	29.4
Ben Davis	28.6	28.2	29.0
Delicious	28.5	28.2	29.1
Grimes Golden	29.0	28.8	29.1
Jonathan	28.3	27.8	28.7
Paragon	28.5	28.5	28.6
Rambo	28.6	28.3	28.9
Stayman Winesap	28.5	28.0	28.9
Winesap	28.2	27.9	28.7
Yellow Newtown	28.0	27.8	28.2
York Imperial	28.3	28.1	28.5
WESTERN GROWN			
Delicious	28.4	28.0	28.9
Gano	28.6	28.3	29.1
Grimes Golden	28.6	28.3	29.1
Jonathan	28.4	28.0	28.7
Rome Beauty	28.9	28.7	29.4
Esopus Spitzenburg	28.7	28.3	29.1
Winesap	28.2	27.9	28.4

The data in Table I show some differences when varieties grown in the same region are compared, and also that the same variety grown in different parts of the country may show a slight variation in its freezing point. In some cases it has been found that the freezing point of a variety differs slightly from year to year with the same strain of apple grown in the same locality. These variations, due to climatic and other environmental differences while the apples are growing, are important in the study of the exact causes and results of freezing injury. The variation of a fraction of a degree does not warrant any change in the storage treatment of the fruit.

UNDERCOOLING AND ITS RELATION TO INJURY

It has been shown (*4*, *5*, *7*, *11*) that plant organisms like potatoes and tomatoes can be undercooled several degrees below their true freezing point, and then warmed again above their freezing point without freezing injury, providing no ice formation takes place within

the tissues. Müller-Thurgau was the first to insist that ice formation in the tissues was a necessary precedent to injury, and this opinion is held quite generally. It seems true, also, that a certain amount of ice formation can occur in apples (*6*) without injury. In other words, there must be a distinction made between apples which are frozen and those which are frozen to death, as apparently there are various degrees of freezing injury in frozen apples.

In the undercooling experiments, apples of several varieties were subjected to low temperatures and left undisturbed, so that undercooling took place. Temperatures were read at intervals until the desired low temperature had been reached, after which the apples were removed and allowed to become warm without ice formation ever having taken place.

TABLE II.—*Data showing temperatures to which apples were undercooled, temperature of the air surrounding the apples, and number of apples showing visual injury*

Temperature to which undercooled	Air temperature at undercooling	Visual injury
WINESAP FROM YAKIMA, WASH.		
°F.	°F.	
23.5	20.7	None.
23.5	20.6	None.
23.5	20.4	None.
23.4	23.0	None.
23.4	23.0	None.
23.0	22.4	None.
22.8	20.4	None.
22.6	20.4	None.
22.6	17.1	None.
22.5	19.6	None.
22.3	19.2	None.
22.2	20.4	None.
22.2	20.4	None.
22.0	20.5	None.
21.8	18.0	None.
21.5	20.5	None.
21.3	20.0	None.
21.2	15.5	(a)
21.0	15.0	None.
20.2	20.0	None.
20.2	20.0	None.
YELLOW NEWTOWN FROM HOOD RIVER, OREG.		
25.0	23.0	None.
25.0	22.6	None.
25.0	22.5	None.
25.0	22.3	None.
25.0	21.3	None.
24.7	23.0	None.
24.0	23.6	None.
24.0	23.0	None.
24.0	22.9	None.
YELLOW NEWTOWN FROM HOOD RIVER, OREG.—Continued		
°F.	°F.	
24.0	22.2	None.
24.0	21.9	None.
23.8	22.8	None.
23.4	23.0	None.
23.4	23.0	None.
23.0	22.4	None.
23.0	22.3	None.
23.0	22.2	None.
23.0	21.4	None.
22.9	22.4	None.
22.6	21.9	None.
22.5	21.9	None.
22.5	21.9	None.
21.8	----------	None.
21.6	----------	None.
20.3	16.5	None.
DELICIOUS FROM OPPORTUNITY, WASH.		
22.2	21.8	None.
22.0	21.8	None.
21.9	21.8	None.
19.8	15.2	(a)
GRIMES GOLDEN FROM ARLINGTON, VA.		
23.7	23.6	None.
23.1	22.3	None.
ROME BEAUTY FROM ARLINGTON, VA.		
22.2	21.4	None.
22.7	21.4	None.

a Some injury not near thermocouple.

With two exceptions, no injury resulted, and in these the damaged area was not immediately adjacent to the thermocouple. Examination of supposedly frozen apples has shown that there may be frozen areas in various parts of the fruit, separated by areas in which apparently little or no ice has formed. If the thermocouple is located in one of the latter, it may not record exactly the condition of ice formation throughout the apple. Though no visual injury was apparent in such apples, changes which could not be detected by careful inspection might nevertheless be taking place. The low temperature to which apples can be undercooled without injury, provided they are not disturbed, is very striking. Inoculation of apples sometimes takes place with comparative difficulty, so it is possible to bring their temperature seven or eight degrees below their freezing points and to hold them there for some time without the formation of ice. Since apples in transit are subjected to motion most of the time, the possibility of any considerable undercooling is slight. As the temperature drops below their freezing points it may be that such apples begin to freeze gradually, possibly by successive inoculations or disturbances producing small increments of ice in the tissues. Such a phenomenon is occasionally observed in apples which are entirely undisturbed, but this involves other causes not now definitely known.

EFFECT OF UNDERCOOLING AS DETERMINED BY THE PRESSURE-TEST METHOD

Since data from other experiments show that one effect of freezing apples is to make them softer and more mealy, apples of two varieties were undercooled and tested with a pressure-testing apparatus similar to that described by Murneek (*8*), which determines the hardness of the fruit. In tests with this apparatus the skin of the apple was removed from an area of about three-quarters of an inch in diameter at three equidistant points about the circumference of the fruit, and the test made of the number of pounds of pressure required to force a smoothly rounded plunger seven-sixteenths of an inch in diameter into the flesh of the fruit for a distance of five-sixteenths of an inch. Tests were also made on the same apples with the skin intact; but it has been found that tests directly on the flesh of the apple are a more accurate index of the real condition of the fruit as regards rela-

tive softness. The skin and its relation to the tissues directly underneath constitute variable factors which may mask the true condition of the apple.

Apples of two varieties, Yellow Newtown and Ben Davis, were used in these tests, 15 of each being brought to undercooling points ranging from 25.1° F. to 25.8° for the Yellow Newtown and from 24.7° to 25.8° for the Ben Davis. The temperature of the freezing room ranged from 22.1° to 22.7°. After undercooling, the apples were removed to a 70° chamber, where they remained for 24 hours, as did also lots of fruit which had not been undercooled. Pressure tests were then made on these lots and on a collateral lot removed directly from cold storage. The freezing points of these two lots of apples were 28.8° for the Yellow Newtown and 29.0° for the Ben Davis.

In undercooled fruit there is apparently no change which can be detected by the pressure-test method. No appreciable differences could be detected between undercooled fruit and that not undercooled, either when the tests were made immediately after undercooling, or after an interval in cold storage following undercooling.

FREEZING INJURY AS VISUALLY DETERMINED

The most obvious results of freezing are discoloration of the tissues of the apples, or the appearance of discolored spots on the surface, and these aspects of injury have received most consideration in the past. They are most important in the diagnosis of freezing injury, although there are other changes of importance in determining keeping qualities and market value after storage.

DIAGNOSIS OF FREEZING INJURY BY VISUAL MEANS.—The diagnosis of freezing injury in apples from the examination of sample specimens, and the determination of previous temperature conditions by such means, present considerable difficulty at times because visual symptoms of freezing injury resemble the so-called physiological breakdown in apples, which is induced by entirely different causes. In such doubtful cases, judgment as to the cause of injury will be modified by any knowledge of the previous history of the fruit. It is essential to consider a number of factors, none of which alone may give certain indication that freezing injury has occurred.

For purposes of rapid diagnosis of freezing injury where hundreds of apples were cut and examined, the severity of the injury was grouped under six heads: None, trace, slight, medium, bad, and very bad. These facilitated estimation of the damage found on cutting the apple, but were themselves classed into larger groups: No injury, trace, or severe injury. The first three headings comprised such fruit as had no injury, or only a relatively unimportant amount. It must be emphasized, however, that visual injury is not the only factor demanding consideration, especially in apples intended for storage, and that apples apparently uninjured may still have undergone changes tending to reduce their storage life.

Slight freezing may occur and leave only a trace of visual injury or none at all, while exposure to very low temperature may so damage the fruit as to destroy its market value. Freezing injury which can be seen may occur in the interior of the fruit and be discovered only upon cutting the apple, or may appear on the surface. These two types of injury may appear independently, or may be found on the same fruit as, for instance, when a severely frozen apple takes on a "baked" appearance. In such a case, the interior may be wholly discolored and mushy, and show other distinctive features which will be described below. In addition, there is an indirect freezing injury due to bruising of the fruit while in the hard-frozen state, the resulting bruises being very much more serious than ordinary bruises.

VISUAL FREEZING INJURY OCCURRING IN THE INTERIOR OF APPLES.—Internal discoloration occurs in two more or less distinct types. In general, when freezing has not been severe and the whole apple tissue is not involved in the breakdown, there is a discoloration of the fibro-vascular system, usually brown, similar to that occurring in frozen potatoes. In cross sections this gives the appearance of brown dots; where a fibro-vascular strand is exposed longitudinally it is seen as a brown line. In Plate 1, A and B, are shown reproductions of water color

EXPLANATORY LEGEND FOR PLATE 1

A.—Cross section of Delicious apple, grown in Washington, showing discoloration of the cortex and secondary fibro-vascular bundles rather characteristic for the variety. The brown portions in the core are probably not freezing injury. A rather severly frozen specimen.

B.—Cross section of Winesap apple, grown in Virginia, showing strong discoloration of the primary fibro-vascular bundles, and also tissue discoloration frequently found in this variety. A rather severely frozen specimen.

A

B

A

B

paintings made from cross sections of a Delicious and a Winesap apple, which are representatives of this type of injury. In Winesap apples, particularly where the secondary fibro-vascular system seems to have a considerable development just beneath the skin, a well-defined net necrosis may often be observed when the skin is peeled off without cutting too deeply into the flesh. Lens examination of thin sections of such apple tissue shows the apple cells generally clear and translucent, and the discoloration in varying tones of brown affecting only the cells of the bundles or those cells immediately adjacent. This fact applies equally to the primary bundles usually seen as large dots in concentric circles around the carpels, and to the secondary bundles scattered through the tissue; but both types of bundles do not necessarily show discoloration in the same apple. This may also be said of discoloration of the cambium, which appears as an entire concentric ring about the carpels and at times seems to join the primary fibro-vascular bundles, as shown in Plate 2, A. Discoloration of all of these may appear in the same apple; in other specimens discolorations may appear alone or in connection with other types of injury.

Discoloration of other interior tissue than the bundles or cambium frequently is found. For some varieties it is evidence that the degree of freezing has been more severe than that producing the injury described above. For others it is the characteristic reaction to any temperature sufficient to produce injury, and here vascular or cambial discoloration may be masked or entirely absent (Pls. 2, B; 3, A and B). This general discoloration of the tissue exhibits all degrees of intensity and when most pronounced marks the severely frozen apple. In some apples, there is in addition a marked softening and a mushy watery appearance; in others the flesh of the apple seems dry and mealy. The tissue resembles that in a state of decay and is wholly discolored, generally in tones of brown, not uncommonly with yellow or green hues. Sometimes quite brilliant blotches of color occur; or clearly defined lighter colored lines radiate from the center; and the cambial region may stand out as a deeply colored area.

VISUAL FREEZING INJURY OCCURRING ON THE SURFACE OF APPLES.—Surface injury other than that due to bruising while frozen consists of discoloration of the surface layers of cells and is usually quite shallow. One of the two usual forms consists of a brown skin discoloration with poorly defined edges resembling a bruise except for a certain water-soaked appearance (Pl. 4, A). This injury is sometimes mistaken for soft scald, but is quite different, because the lesions of soft scald are definite in outline, dull in color, and somewhat sunken in a late stage. The other common form of surface injury occurs only on red apples or on the blushed side of the fruit. (See Pl. 4, B.) It is a yellowish-brown discoloration resembling in some cases the work of leaf-miner larvae in leaves. More often it appears as small discolored areas, very irregular in outline but rather clearly defined. In storage these areas may remain quite intact, or they may become more or less sunken, enlarged, and of a dark brown color. This enlargement seems caused by death and discoloration of cells adjacent to those showing injury very soon after freezing has taken place. In many Winesap apples the tissue immediately beneath such places had dried completely after a storage period of three months, and had been replaced by cavities sometimes 10 mm. in depth, lined with brown corky tissue composed of dead cells. To some degree these cavities resemble the drought cracks occasionally found in apples like the York Imperial, and probably are caused by an increased water loss from the frozen tissues.

Since the intercellular spaces have intimate connection with the lenticels or corky openings in the skin, the loss of water vapor is augmented above the ordinary amount. The greater tendency to wilting and to rapid water loss is characteristic of frozen apples thawed under conditions favoring the removal of moisture from the fruit. Apples frozen to a certain degree may also have a water-soaked appearance, and a skin tough and resilient to the touch. As a general rule, surface discoloration due only to freezing is very shallow. In varieties with a green color, notably the Yellow Newtown, it often occurs before other visual freezing injury can be de-

EXPLANATORY LEGEND FOR PLATE 2

A.—Cross section of Grimes apple, grown in Virginia, showing discoloration of the carpel and cambial regions, and of the primary fibro-vascular bundles. A rather severely frozen specimen.

B.—Cross section of Ben Davis apple, grown in Virginia, showing discoloration of the central portion of the fruit. The brown areas near the periphery are probably bruises made while the fruit was in a hard frozen condition. A rather severely frozen specimen.

tected, and detracts from the appearance of the fruit, injuring its market value. Yellow Newtown apples so affected may appear to have been subjected to very severe bruising while in the hard frozen state.

TIME OF APPEARANCE OF VISUAL INJURY IN FROZEN APPLE TISSUE.—The discoloration of apple tissue which follows a certain degree of freezing is generally not visible while the fruit is still frozen. As thawing proceeds, it becomes more and more evident. Where freezing is not severe it may increase in degree, and to a lesser extent in area, as the ice in the tissue melts (Table III).

TABLE III.—*Effect on Yellow Newtown apples (in lots of 10) of varying periods of exposure to 15° F., and of different periods of storage at 40° and 65° after freezing*

Period of exposure	Number of apples showing injury in—			
	5 hours	8 hours	13 hours	90 hours
Hours	SUBSEQUENT STORAGE AT 65° F.			
3	0	0	0	4
6	2	2	[a] 4	[a] 5
25	10	10	10	10
47	10	10	10	10
73	10	10	10	10
	SUBSEQUENT STORAGE AT 40° F.			
3	0	0	[a] 1	[a] 2
6	0	[a] 1	[a] 2	[a] 5
25	[a] 3	[a] 3	[a] 7	10
47	0	[a] 5	10	10
73	0	[a] 7	10	10

[a] Slight injury only.

From Table III it is evident that the severity of the injury to Yellow Newtown apples exposed for the shorter periods increases with the length of the storage period. It seems improbable, however, that it increases materially when the ice in the tissue has entirely melted; where such increase occurs it may be due to secondary influences in tissues weakened by freezing rather than to killing by the formation of ice. Thus it is apparent that when moderate freezing injury has occurred, it is impossible to determine accurately the amount of injury until a considerable interval of time has elapsed following thawing.

The term "baked" or "cooked" is frequently given to very severely frozen apples which break down completely, actually presenting the brown, wrinkled appearance of an apple subjected to high temperature. Such surface injury is readily recognized.

EFFECT OF BRUISING FROZEN APPLES

The bruising of hard frozen apples results in a much more serious injury than that produced by similar bruising of unfrozen fruit. Some experiments were made to determine in what respects such bruises made while the fruit was frozen differed from the ordinary bruises; whether the freezing of bruises already present changed their appearance and depth; and whether the temperature of thawing had an effect on the seriousness of the bruise as measured by its depth.

The apple to be bruised was held on its side on the platform of a weighing scale having a capacity of 50 pounds. A flat surface was brought in contact with the upper side of the fruit and pressure applied until the pointer on the dial recorded the desired figure in pounds. The apple was then turned and pressure again applied, thus producing four bruises fairly equally spaced about the circumference. While this does not give exactly the type of bruise encountered where the fruit has been subjected to jolting or bumping or has received sudden sharp blows rather than more prolonged pressure, it was found satisfactory for the investigation of certain features of this problem. After further storage or treatment, the apple was cut across the middle horizontally and the depth of the bruises determined in millimeters. The greatest distance to which the discoloration had penetrated was taken as the depth of the bruise. The sample lots consisted of 10 apples each having 4 bruises, and the data presented in Table IV are, in each case, averages of the maximum depth of 40 bruises.

DIFFERENCES IN APPEARANCE OF BRUISES ON HARD FROZEN AND ON UNFROZEN APPLES.—Bruises made on apples while frozen differ in several respects from those on unfrozen fruit. The bruises in hard frozen apples have a greater depth and are conical in shape with the apex reaching nearer to the

EXPLANATORY LEGEND FOR PLATE 3

A.—Cross section of Yellow Newtown apple, grown in Washington, showing extensive discoloration involving the tissue cells, and rather characteristic of this variety. A bruise discoloration is found near the periphery. A rather severely frozen specimen.

B.—Cross section of Grimes apple, grown in Virginia, showing extensive tissue-browning as well as discoloration of the cambial and carpel regions. A severely frozen specimen.

A

B

A

B

middle of the fruit than on apples similarly bruised while unfrozen. Bruises in frozen apples have a more indefinite outline and a more complete wrecking of the tissue organization as shown by the character of the discoloration.

In the ordinary bruises in unfrozen fruit lines of cleavage tend to appear in the bruise approximately parallel to the surface, and these are often separated by strips of apparently uninjured tissue. This feature is never found in the bruise made on hard-frozen fruit. It may be that, whereas pressure on unfrozen tissue causes the cells to separate along the weakest lines, similar pressure on hard-frozen tissue with the intercellular spaces filled with ice crystals might entirely crush and rupture the tissue organization. Examined through a hand lens by transmitted light, thin sections of bruised tissue on hard-frozen fruit show many clear translucent areas. When surrounded by discolored tissue they appear brown by reflected light. They apparently represent portions of the tissue in which the ice crystals completely obliterated the cell structure, and the cavities filled with water when the ice melted. The ordinary bruise in cross section has a dry, corky appearance while the bruise made on hard-frozen fruit appears more watery.

The surface appearance of the bruise before the apple is cut will sometimes give a clue to the conditions under which the bruise occurred, that made on frozen fruit having a more water-soaked appearance and being softer and less resistant to pressure by the fingers than a bruise in unfrozen fruit. Old bruises on fruit which was afterwards frozen do not differ appreciably in appearance from the ordinary bruises on apples not subsequently exposed to freezing temperatures. Plate 5, A and B, shows bruises made on frozen and unfrozen Yellow Newtown apples. Examination of the bruises is oftentimes a very great aid to the diagnosis of freezing injury.

EFFECT OF BRUISING WHILE HARD FROZEN.—Bruises were made on apples of different varieties while hard frozen, unfrozen lots bruised with exactly th same pressure being measured or comparison. The apples were fro en by exposing them 24 hours to tempe atures around 22° F., and were thaw by placing them at 68° for another 24-hour period (Table IV). It will be seen that the injury in general, as measured by the depth of the bruise was about twice as severe in apples bruised when hard frozen as in unfrozen apples. The damage to their appearance is much more serious, and likely to cause marked depreciation in their market value.

TABLE IV.—*Comparison of the depth of bruises in frozen and unfrozen apples, with pressure applied for bruising*

Variety	Pressure applied for bruising	Bruise depth		Ratio of depth of frozen to unfrozen bruises
		Unfrozen	Hard frozen	
	Lbs.	*Mm.*	*Mm.*	
Baldwin	50	7.3	14.1	1.9
Rome Beauty	30	6.0	14.2	2.4
	50	9.5	17.5	1.8
Esopus Spitzenburg	50	5.1	15.3	3.0
Winesap	50	6.3	14.0	2.2
Yellow Newtown	30	6.7	16.8	2.5
York Imperial	50	6.2	12.8	2.1

EFFECT OF THE PRESENCE OF DIFFERENT AMOUNTS OF ICE UPON THE SEVERITY OF INJURY FROM BRUISING.—The presence of ice is presumably the cause of augmentation of injury when hard-frozen fruit is bruised. Tests were made with apples which were hard frozen and then bruised in different stages of thawing. Comparisons were made with lots of apples bruised while hard frozen and also with those bruised when unfrozen (Table V).

Damage by bruising is obviously lessened as the ice in the tissues melts. When it has entirely disappeared, bruising of the tissues produces injury only slightly more severe than that caused by a similar bruising of unfrozen fruit, at least so far as concerns the depth of the injured tissue. Tests made with other varieties than Rome Beauty gave similar results, indicating that frozen apples should be allowed to thaw before they are moved. The bruises suffered in handling will then be, at the most, only slightly more serious in depth and character than if the fruit had not been frozen at all.

EXPLANATORY LEGEND FOR PLATE 4

A.—Surface view of Yellow Newtown apple, grown in Washington, showing epidermal discolorations rather characteristic of green-skinned varieties and especially Yellow Newtown, and not necessarily caused by bruising.

B.—Surface view of Delicious apple, grown in Washington, showing epidermal discoloration rather characteristic of red-skinned varieties.

However, there is some indication that frozen apples stored undisturbed for an extended period in the original position after thawing will suffer somewhat deeper and more serious bruises when moved than unfrozen fruit similarly stored. Freezing, even if unaccompanied by visual injury, tends to soften the fruit, making it less resistant to injury after an extended storage period. But the severity of injury is not nearly so great as in fruit bruised while hard frozen.

TABLE V.—*Comparison of the effect of bruising unfrozen and partially frozen Rome Beauty apples as shown by the depth of the injured portion when thawed*

Treatment	Bruise depth	Ratio of depth of frozen to unfrozen bruises
30 POUNDS PRESSURE APPLIED FOR BRUISING		
	Mm.	
Unfrozen	6.0	
Bruised hard frozen	14.2	2.4
Thawed 1 hour at 68° F	13.4	2.2
Thawed 3 hours at 68° F	8.0	1.3
Thawed 5 hours at 68° F	7.8	1.3
Thawed 7 hours at 68° F	7.7	1.3
Thawed 24 hours at 68° F	7.0	1.2
Thawed 7 hours at 40° F	10.9	1.8
Thawed 24 hours at 40° F	7.5	1.3
Thawed 48 hours at 40° F	6.6	1.1
50 POUNDS PRESSURE APPLIED FOR BRUISING		
Unfrozen	9.5	
Bruised hard frozen	17.5	1.8
Thawed 1 hour at 68° F	17.4	1.8
Thawed 3 hours at 68° F	12.3	1.3
Thawed 5 hours at 68° F	12.9	1.4
Thawed 7 hours at 68° F	12.0	1.3
Thawed 24 hours at 68° F	10.3	1.1
Thawed 7 hours at 40° F	14.6	1.5
Thawed 24 hours at 40° F	11.0	1.2
Thawed 48 hours at 40° F	10.7	1.1

EFFECT OF FREEZING ON DEPTH OF BRUISES ALREADY PRESENT.—Yellow Newtown apples were used in experiments to obtain data upon the effect of freezing apples which were already bruised. The fruit was subjected to temperature and handling conditions similar to those followed in the other bruising experiments, but before being frozen it was stored for certain periods after bruising (Table VI).

TABLE VI.—*Effect of freezing upon depth of bruises previously made with 30 pounds pressure on Yellow Newtown apples*

Treatment after bruising	Storage temperature after bruising	Length of storage period	Bruise depth
	°F.	*Days*	*Mm.*
Frozen	70	1	6.7
Unfrozen	70	1	6.0
Frozen	70	3	6.6
Unfrozen	70	3	6.6
Frozen	70	6	6.1
Unfrozen	70	6	6.1
Frozen	40	14	6.0
Unfrozen	40	14	6.0

There is no indication that bruises made before the fruit was frozen become more serious in depth when the fruits are subsequently frozen, nor that they change in appearance. Tests with other varieties gave similar results.

EFFECT OF DIFFERENT THAWING TEMPERATURES ON DEPTH OF BRUISES ON HARD FROZEN FRUIT.—To determine what effect the temperature at which fruit is held while thawing has on the injury from bruises, a number of Esopus Spitzenburg apples were frozen hard, and bruised as already described. The fruit was then thawed at various temperatures from 32° to 70° F.

No significant differences appear in the depth of bruises made on hard-frozen fruit when it is subjected to various thawing temperatures (Table VII).

DETERMINATION OF THE INTERNAL TEMPERATURE AT WHICH VISUAL INJURY TAKES PLACE IN FROZEN APPLES

Certain peculiarities in the reaction of apples to supposedly fatal temperatures have been noted in reading the temperature by means of the thermocouples. Apples quite similar in weight and appearance show marked variations in the rate of cooling and of freezing, and exhibit the most diverse phenomena in visual injury. Experiments were made to test the hypotheses, repeatedly noted, that ice formation is not in itself necessarily injurious to the fruit but that apparently a certain

EXPLANATORY LEGEND FOR PLATE 5

A.—Cross section of Yellow Newtown apple, bruised while unfrozen by the application of 35 pounds of pressure to areas on its surface.

B.—Cross section of Yellow Newtown apple, bruised while hard frozen by the application of 35 pounds of pressure to areas on its surface, showing the severity made on hard-frozen tissue.

A

B

amount of ice formation is required before injury results. Freezing points of a large number of apples of a certain variety were determined. When this was accomplished the apples were allowed to remain in the freezing room and further records were made of their internal temperatures.

TABLE VII.—*Effect of different thawing temperatures on the depth of bruises made with 50 pounds pressure on hard frozen Esopus Spitzenburg apples*

Condition when bruised	Thawing temperatures	Duration of exposure to thawing temperatures	Bruise depth	Ratio of depth of frozen to unfrozen bruises
	° *F.*	*Hours*	*Mm.*	
Unfrozen	70	24	5.1	-------
Frozen	70	24	15.3	3.0
Do	70	48	15.0	-------
Unfrozen	70	72	5.0	-------
Frozen	70	72	16.8	3.4
Unfrozen	50	24	5.1	-------
Frozen	50	24	15.4	3.0
Do	50	48	14.0	-------
Unfrozen	50	72	6.0	-------
Frozen	50	72	16.2	2.7
Unfrozen	40	24	5.5	-------
Frozen	40	24	15.0	2.7
Do	40	48	19.3	-------
Unfrozen	40	72	5.1	-------
Frozen	40	72	14.9	2.9
Unfrozen	32	24	5.1	-------
Frozen	32	24	14.3	2.8
Do	32	48	13.4	-------
Unfrozen	32	72	5.1	-------
Frozen	32	72	14.8	2.9

The internal temperature in an apple exposed to freezing conditions usually drops below the freezing point, that is, it is undercooled. If the temperature is reduced enough, ice crystals begin to form, and the heat of fusion produced in the formation of the ice crystals raises the temperature of the apple to a point where it remains practically stationary, or with only slight reduction, until part of the water in the tissues which can be frozen out in the form of crystals of ice has been thus changed. The point at which the temperature remains constant is called the freezing point. After this is reached the temperature of the apple gradually becomes lower and approaches that of the room or environment of the fruit, where it can be held almost indefinitely after some minor fluctuations.

These fluctuations may be due to the further crystallization of small amounts of water which are forced from the liquid state by the increasing internal tension in the cells of the apple as the temperature continues to fall. Figure 1 shows a typical curve of the freezing of a Yellow Newtown apple, and illustrates the points mentioned above. A portion of the curve showing the temperature drop while the fruit was being cooled from the storage temperature of 34° F. has been omitted for the sake of brevity. The reactions of the fruit noted during and after the freezing process suggest that visual injury occurs when the temperature drops a certain distance below the freezing point. This injury is probably due to the loss of water from the protoplasm of the cells, which has been shown to occur during the freezing process. In other words, the internal temperatures measured as freezing goes on are an indirect measure of the amount of ice which has been formed; and this degree of ice formation may determine the occurrence of visual injury for any set of internal conditions in the fruit. It will be shown elsewhere that other changes also seem to occur in the fruit as soon as ice formation has taken place.

To obtain further information on the internal temperatures of frozen apples at which visual injury occurs, readings of the freezing point and of the internal temperatures when the fruit was removed from the freezing room have been collected, together with other relevant data. These are so voluminous that they are presented only in brief summary form to bring out certain facts (Table VIII).

The temperatures prevailing in the freezing room while the different lots were under observation did not vary widely for the three varieties. The maxima and minima were 22.3° F. and 21° for the Ben Davis, 22.7° and 21.9° for the Winesap, and 22.4° and 21.3° for the Rome Beauty. All the apples in this experiment were grown on Arlington Experiment Farm, Rosslyn, Va.

In the case of the frozen Ben Davis apples studied, Table VIII indicates in the average only a small amount of visual injury when the internal temperatures were 4° F. below the freezing point. Below this point the percentage of injury increased rather sharply, though not markedly, so that in the group where the temperature was 6.1° to 7° below the freezing point nearly two-thirds of the apples showed visual injury. No injury appeared in 14 apples of this variety when the internal temperatures dropped from 3.1° to 4° below their freezing points.

The internal temperature of frozen Winesap apples could not go so far below their freezing point without

TABLE VIII.—*The amount and degree of visual injury occurring in Ben Davis, Winesap, and Rome Beauty apples when their internal temperatures fall to different temperatures below their freezing point after freezing has taken place*[a]

Difference between freezing-point temperature and temperature at removal from freezing conditions, in groups of 1° F.	Freezing points			Visual injury in per cent			Total number of apples
	Average	Maximum	Minimum	None	Trace	Severe	
BEN DAVIS							
3.1–4.0° F.	28.8	29.1	28.6	100.0	0	0	4
4.1–5.0° F.	28.8	29.2	28.1	65.5	30.7	3.8	26
5.1–6.0° F.	28.9	29.4	27.8	36.9	44.7	18.4	65
6.1–7.0° F.	28.9	29.4	28.2	37.4	39.7	22.9	83
WINESAP							
2.5–3.4° F.	28.6	28.9	28.1	75.0	20.8	4.2	24
3.5–4.4° F.	28.3	29.0	27.6	62.5	25.0	12.5	8
4.5–5.4° F.	28.4	29.0	27.8	12.5	75.0	12.5	40
5.5–6.4° F.	28.6	29.0	27.9	0	40.5	59.5	37
ROME BEAUTY							
2.1–3.0° F.	29.5	29.9	29.0	70.6	29.4	0	17
3.1–4.0° F.	29.4	30.0	28.5	73.3	23.4	3.3	30
4.1–5.0° F.	29.1	29.8	28.3	90.7	7.0	2.3	43
5.1–6.0° F.	29.2	29.8	28.4	64.4	28.7	6.9	73
6.1–7.0° F.	29.2	29.7	28.0	30.9	42.8	26.3	42
7.1–8.0° F.	29.3	29.7	28.4	13.7	40.9	45.4	22

[a] The differences in temperature between the freezing point and the removal temperature of the apples, calculated to tenths, have been condensed into groups of 1° F. each. For instance, all the temperature differences of a magnitude from 3.1° and 4.0° appear in the group so designated.

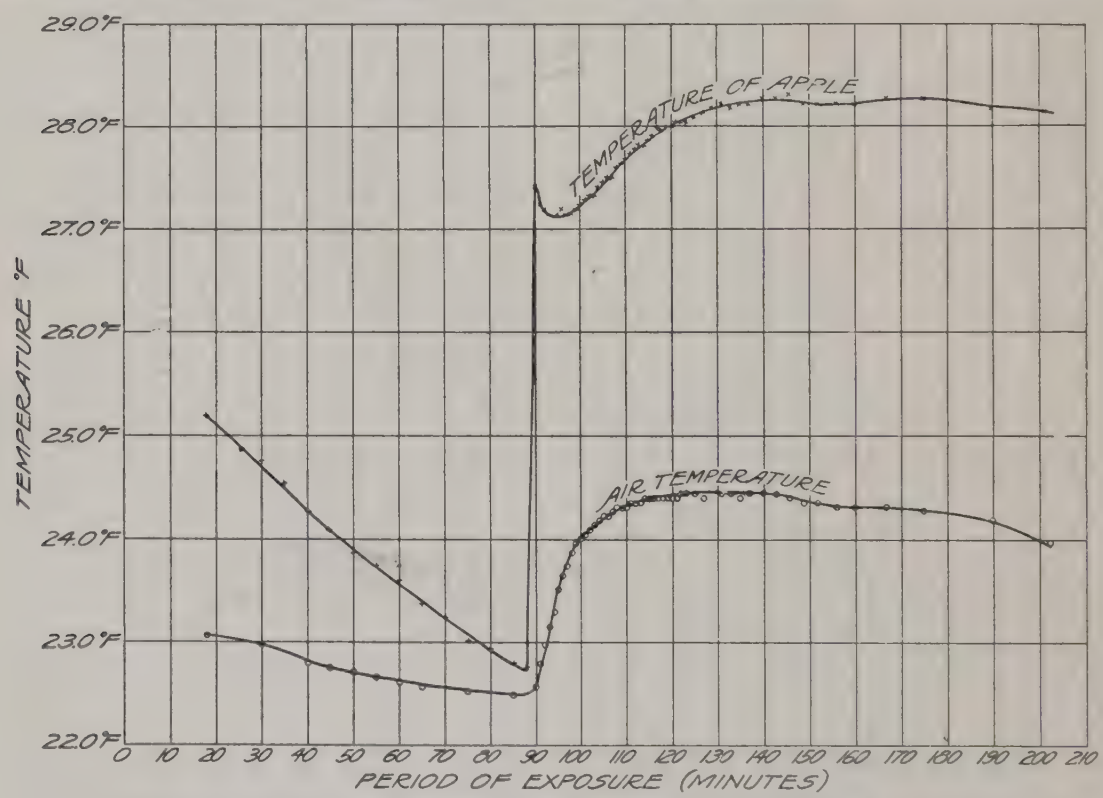

FIG. 1.—Internal temperature of a freezing Yellow Newtown apple exposed to low temperature conditions. Temperature of the air surrounding the fruit also given

injury. Even in the group in which the temperatures were between 2.5° F. and 3.4° below the freezing point, 25 per cent of the apples were injured. The number of injured apples and degree of injury increase greatly as the internal temperature drops farther below the freezing point.

Frozen Rome Beauty apples showed about 30 per cent slight injury when the internal temperature was only 2° to 3° below the freezing point. A temperature of 6° to 8° below the freezing point caused very severe visual injury.

Apparently there is a marked variation in the temperatures which different varieties of apples will endure without visual injury. Ben Davis will stand exposure to distinctly lower tempera-

tures than will Winesap and Rome Beauty. Under the conditions of this test, actual internal temperatures of 25° F. or lower were required to injure Ben Davis. An internal temperature of 26° resulted in some injury to Winesap, while 27° was sufficient to result in slight injury to Rome Beauty. Below these temperatures, injury increased markedly with successive decreases in temperature. The lower the temperature attained, the greater the quantity of ice formed in the tissues and the greater the apparent injury.

These temperatures represent about the range at which visual injury will first occur when the apples thaw rather slowly in air temperatures. Certain data subsequently secured indicate that with extremely rapid thawing, such as that induced by immersing the frozen apples in warm water, visual injury may occur at somewhat higher temperatures. Internal temperatures even one degree below the freezing point may result in injury if followed by extremely rapid thawing. For commercial conditions, however, where thawing would always be in air, actual fruit temperatures of 25° to 27° represent about the highest point at which visual injury may be expected to occur.

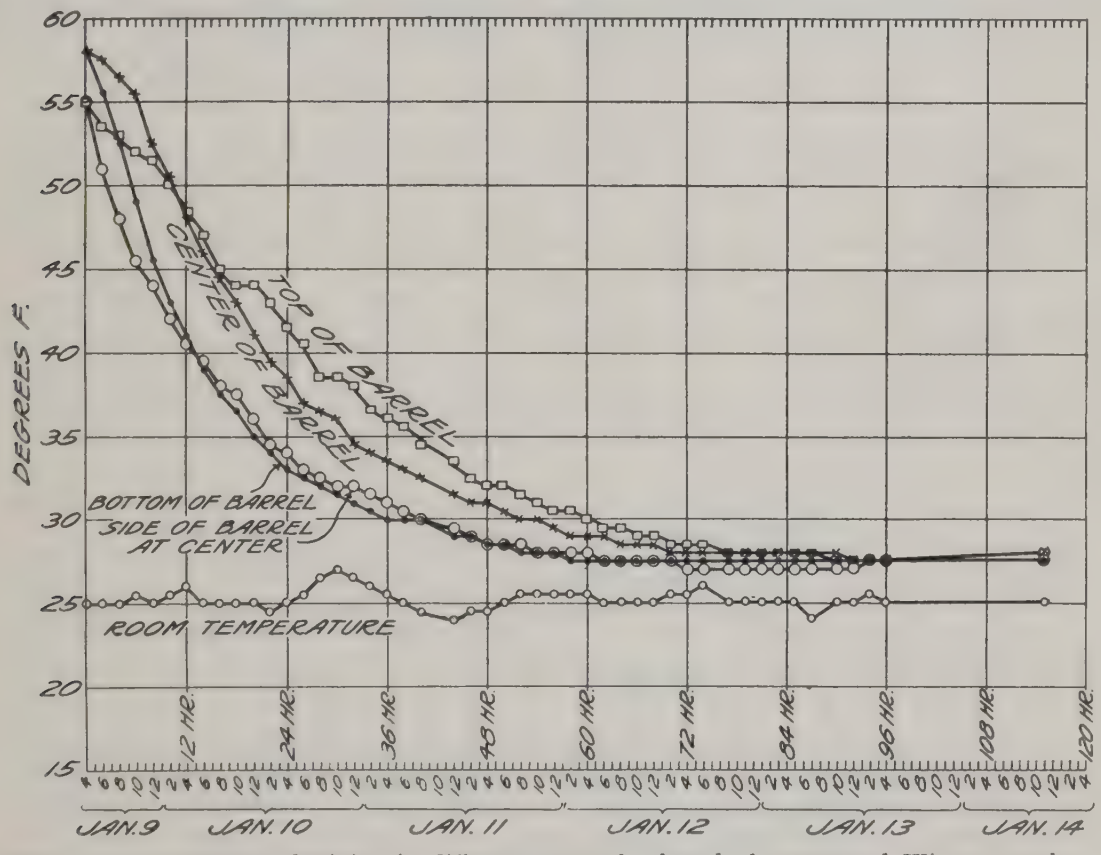

FIG. 2.—Temperatures obtaining in different parts of a barrel of unwrapped Winesap apples during exposure to external temperatures around 25° F. for 120 hours

THE RATE OF COOLING DOWN OF BARRELS AND BOXES OF APPLES AT DIFFERENT FREEZING TEMPERATURES

It is reasonable to suppose that temperature curves showing the rate of cooling of fruit packed in various types of containers would differ under constant low temperature conditions outside the package. To obtain more evidence on this point, Winesap and York Imperial apples were exposed to freezing temperatures in barrels and in commercial apple boxes. The barrels were placed on end and the boxes were laid horizontally on a wooden floor rack 6 inches above the floor of the room. Part of the boxed apples were wrapped and part unwrapped. One series was run at about 25° F. and another at about 20°. The reduction in temperature within the packages was read by thermocouples every two or three hours during at least four or five days, and in some instances this was carried on at longer intervals to 12 days. In this way a complete history was secured of temperature changes in the fruit in different portions of the packages and in the surrounding air. The graphs presented in Figures 2 to 12 show the temperature changes occurring in the apples.[5]

[5] The preparation of the packages for these experiments was as follows: Two thermocouples were inserted in apples in each of the following locations in the packages: Barrel: Top, center, side at center and bottom. Boxes of wrapped and unwrapped apples: Top, center, and bottom. The two readings from the same location were averaged for better comparison of the data.

The temperature curves for Winesap apples packed in a barrel are presented in Figure 2. The room temperature was kept as near 25° F. as possible although some unavoidable variations occurred. It will be seen that the temperatures in all parts of the barrel drop rather rapidly at first, and when freezing begins the curves flatten out and the temperatures remain fairly constant for considerable periods of time with wholly insignificant variations. Undoubtedly this is the freezing point of the apples, not only a somewhat composite one for the particular fruit in which the thermocouple is inserted, but one influenced by the fact that adjacent apples are also

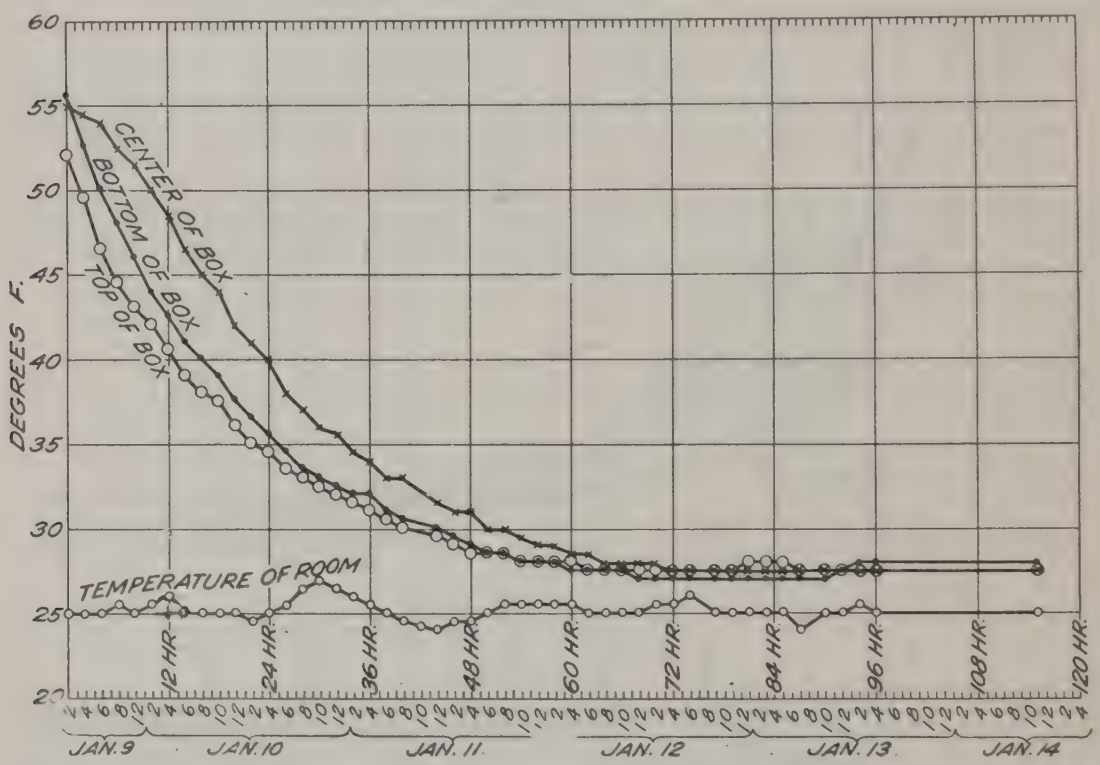

FIG. 3.—Temperatures obtaining in different parts of a box of wrapped Winesap apples during exposure to external temperatures around 25° F. for 120 hours

freezing. The heat of fusion produced by ice crystallization in these apples diffuses into the air spaces between them, mutually affecting the heat gradients existing between the exterior and interior tissues. Upon this heat gradient, which is itself a complex of other factors, depend the rate of further crystallization, the path of temperature curves, and even the injury and death of the tissues. It is a problem of great complexity and can be but briefly considered here.

It should be noted that there is no evidence of undercooling in the path of the temperature curves for the apples in the barrel. This does not mean that individual apples do not undercool under such conditions, for there is much reason to assume that they do. The visual injury to the fruit used in these experiments was not quantitatively determined; but apples with severe visual injury were found throughout different parts of the barrel. Probably these apples froze comparatively early in the period of exposure, since it has been shown that in general there must be a rather considerable temperature difference between their freezing point and the temperature at which injury commences. It is suggested that the heat of fusion given off by the early ice crystallization in these scattered apples may be sufficient to raise the temperature of any particular barrel location so as to delay the time of ice formation in other apples. In other words, some apples may be frozen, and the heat they give off during the process tends to delay the freezing of others.

The paths of the temperature curves for apples exposed in packages to low temperature generally show comparatively little undercooling before freezing commences. At any rate it has not been possible to carry the undercooling to the degree to which it has been shown to occur in isolated apples, surrounded on all sides by cold air, when these are not disturbed.

The data show further that the bottom and sides of the barrel cool most quickly, the top remains warm longest, and the center fruit in the barrel is intermediate in the rate of cooling. This is logical, since the heated air

naturally rises to the top, while the colder air being heavier, tends to settle into the bottom of the barrel. In addition, there is also heat conduction through the package, irrespective of convection currents within. A study of the results shows that it was two days before the bottom and sides of the barrel showed a steady temperature indicative of the freezing point, although some freezing undoubtedly occurred before that time. For fruit at center and top of the barrel another day passed before the freezing process was well under way.

The data for the boxed wrapped apples are presented in Figure 3, those for the boxed unwrapped apples in Figure 4. The fruit in these boxes was not packed according to any commercial pack, though regular commercial wraps were used. The general trend of the two groups of curves is strikingly different while the temperature is dropping to the freezing point of the fruit. The temperatures in the wrapped apples show a gradual decline, while those in the unwrapped apples show a sudden drop with some suggestion that undercooling took place previous to freezing. These differences will be brought out very clearly if the position of curves is noted at the same time periods. Wrapping the fruit noticeably retards heat loss when the package is exposed to freezing temperatures.

In order to show this comparative difference more easily, the temperature curves for the apples from the center of the barrel and from the center of the two boxes were plotted together and are given in Figure 5. The curves for the wrapped apples and the fruit in the barrel almost coincide. In one instance, the wrappers retarded heat loss from the center, in the other a greater amount of fruit intervening between the center and the exterior served a similar purpose. As the curve for the unwrapped apples differs considerably from the other two curves, it is especially interesting to compare the behavior of the two boxes of fruit. The interior temperature of the wrapped apples indicates that freezing began in amounts sufficient to offset the heat losss to the exterior in about 64 hours. In the unwrapped apples it seems to have begun in such amounts in 32 hours, showing that wrapping the fruit retarded the beginning of freezing nearly a day and a half.

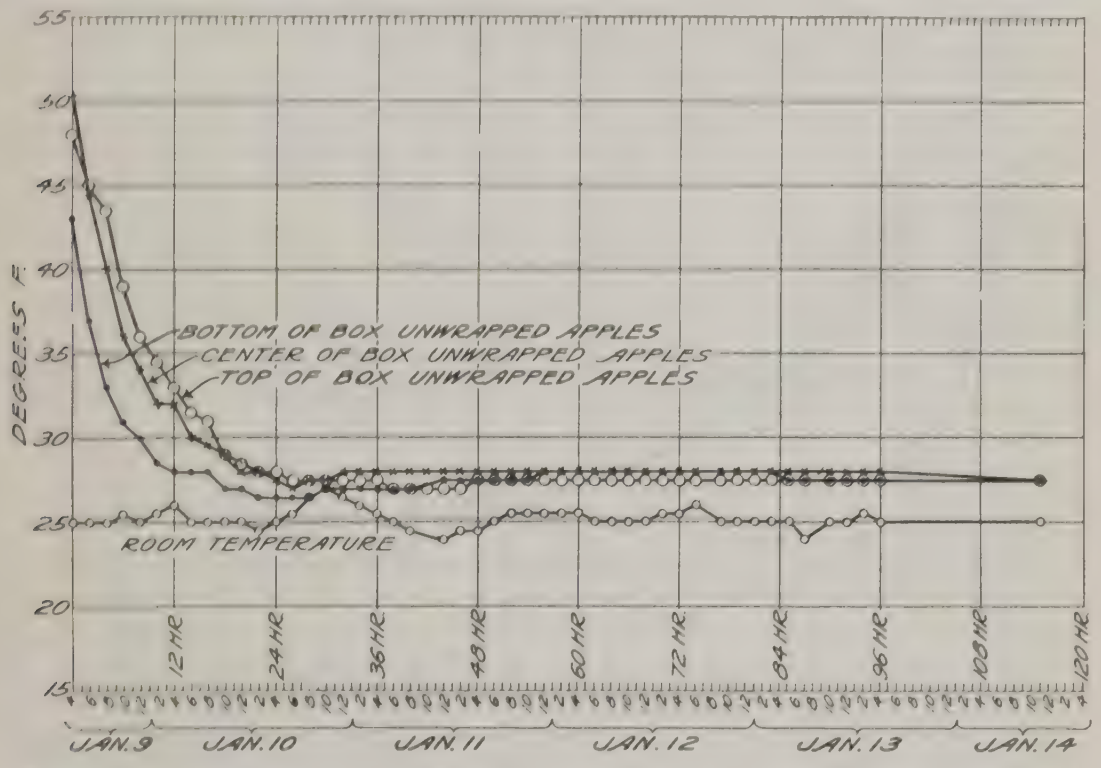

FIG. 4.—Temperatures obtaining in different parts of a box of unwrapped Winesap apples during exposure to external temperatures around 25° F. for 120 hours

In Figure 3 there is an apparent discrepancy in the relative position of curves for the top, center, and bottom, in that the apples in the top of the box seem to have been the coldest. The apples in this experiment were wrapped and packed in the freezing room in order to place the thermocouples in their proper position. Since the apples in the top of the box were packed last, they were exposed unwrapped to

the low temperatures for the longest time. Consequently their initial temperature was lower and continued so. There seems to have been no considerable warming of the fruit in the upper layers by heat given off from apples in the interior of the box. The data are plotted in Figure 3 for only 120 hours, but the experiments were carried on for 12 days. At the end of this time the maximum drop from the freezing point in the bottom of the box of unwrapped fruit was only 1.5° F. In some instances, as for example the center of the box of wrapped apples, the temperature reading at 12 days was practically the same as it had been at 64 hours, indicating that ice formation in the fruit had been going on with the production of heat of fusion in amounts sufficient to offset the heat loss to the colder exterior. In nearly all cases, the temperature drop at the end of 12 days was a fraction of a degree below the temperature at which the fruit had been for a long time and which was apparently the freezing point.

Figures 6 and 7 present the data for the York Imperial apples packed and handled similarly to those of the Winesap variety. The graphs for the temperature changes in the fruit in the barrel closely resemble those shown in Figure 2. This is true also of the graphs for temperature changes in the boxes of wrapped and unwrapped Winesap apples compared with similarly handled York Imperial apples. No temperature readings were made in the centers of boxes of the latter. The data given in Figure 7 are for both wrapped and unwrapped York Imperial apples. It should be noted that the discrepancy in position of the temperature graph for the top of the box of wrapped Winesap apples is found only for a short time in the case of the York Imperial, after which the heat from the center of the box probably warmed the upper layers so that the temperature of the top of the box thereafter was higher than that of the bottom.

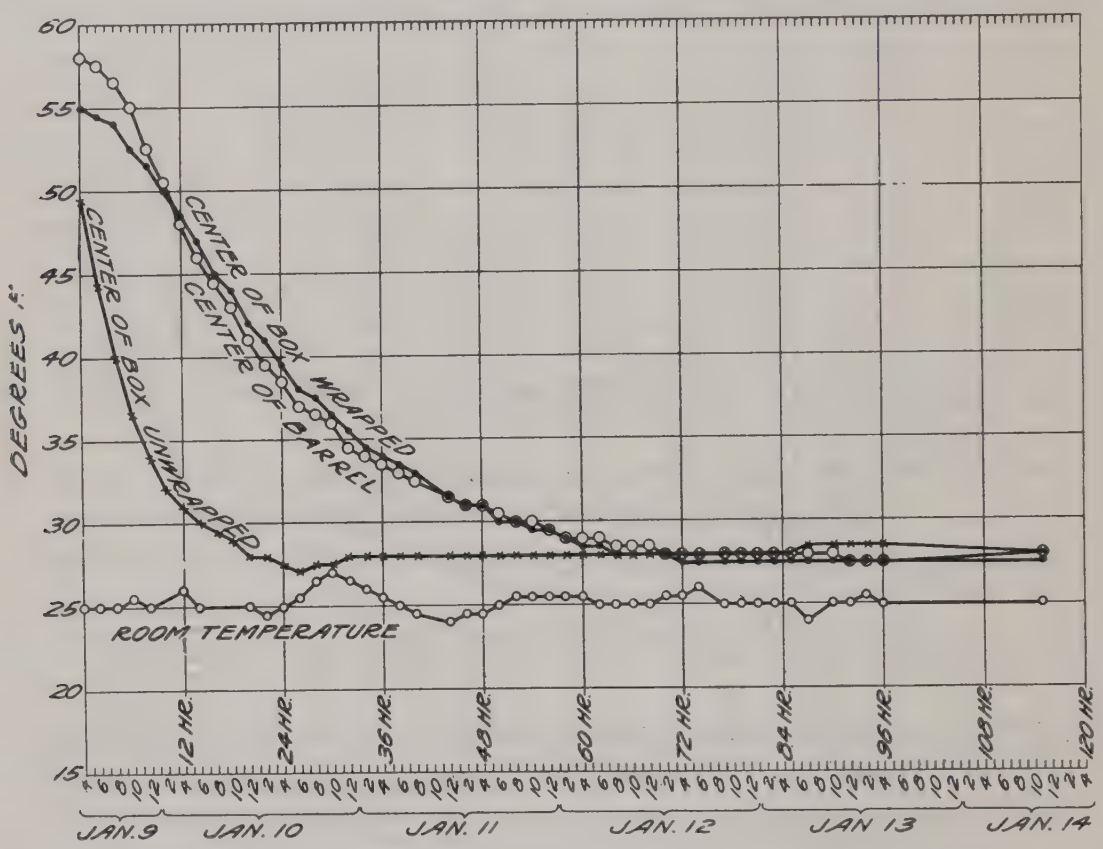

FIG. 5.—Comparison of the temperatures obtaining in the center of the box of wrapped, in the box of unwrapped, and in the barrel of unwrapped Winesap apples during exposure to external temperatures around 25° F. for 120 hours

Another series of experiments was carried on with a freezing-room temperature at or below 20° F., with general results similar to that at 25° F., the important difference being that the interior temperature of the packages drops more rapidly at the lower freezing temperature. Figures 8 and 9 give the results obtained for the two barrels of apples, Winesap and York Imperial, for a period of 96 hours. The fact that during the first few readings fruit in the top of the barrel is apparently colder than fruit in the center or at the bottom of the barrel is probably due to inci-

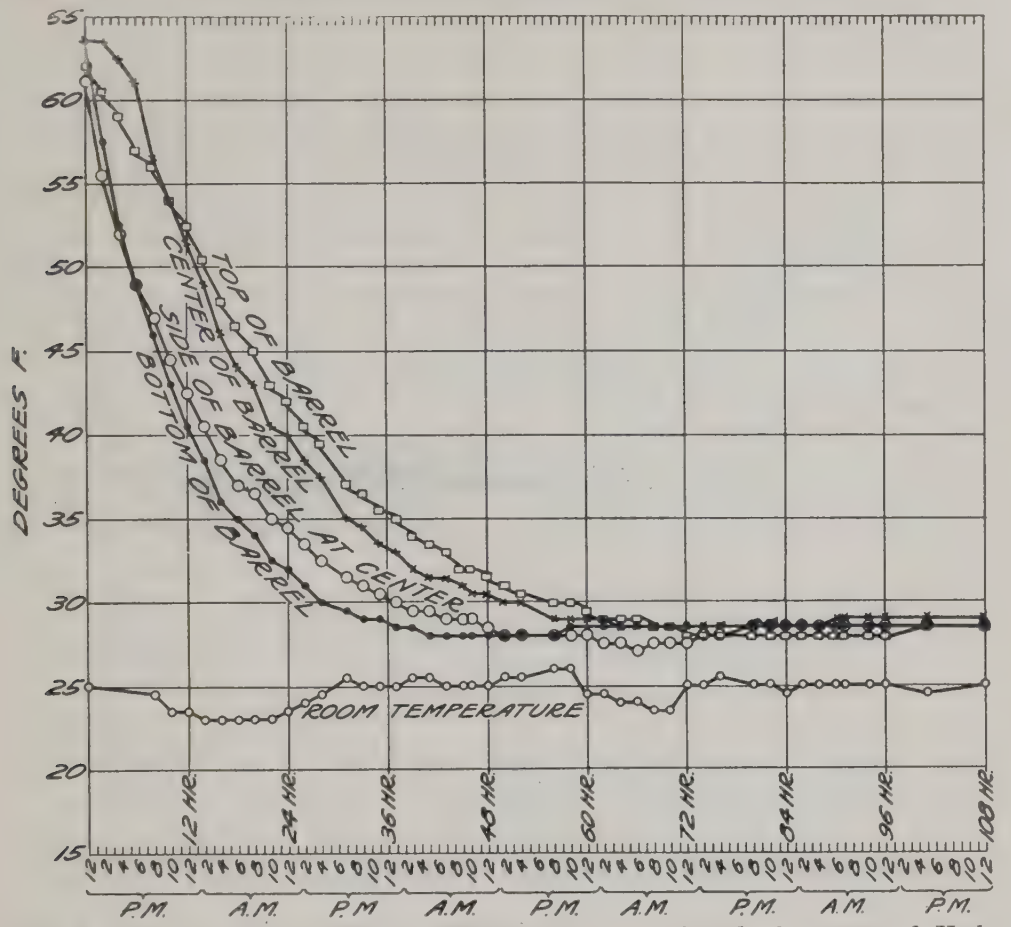

FIG. 6.—Temperatures obtaining in different parts of a barrel of unwrapped York Imperial apples, during exposure to external temperatures around 25° F. for 120 hours

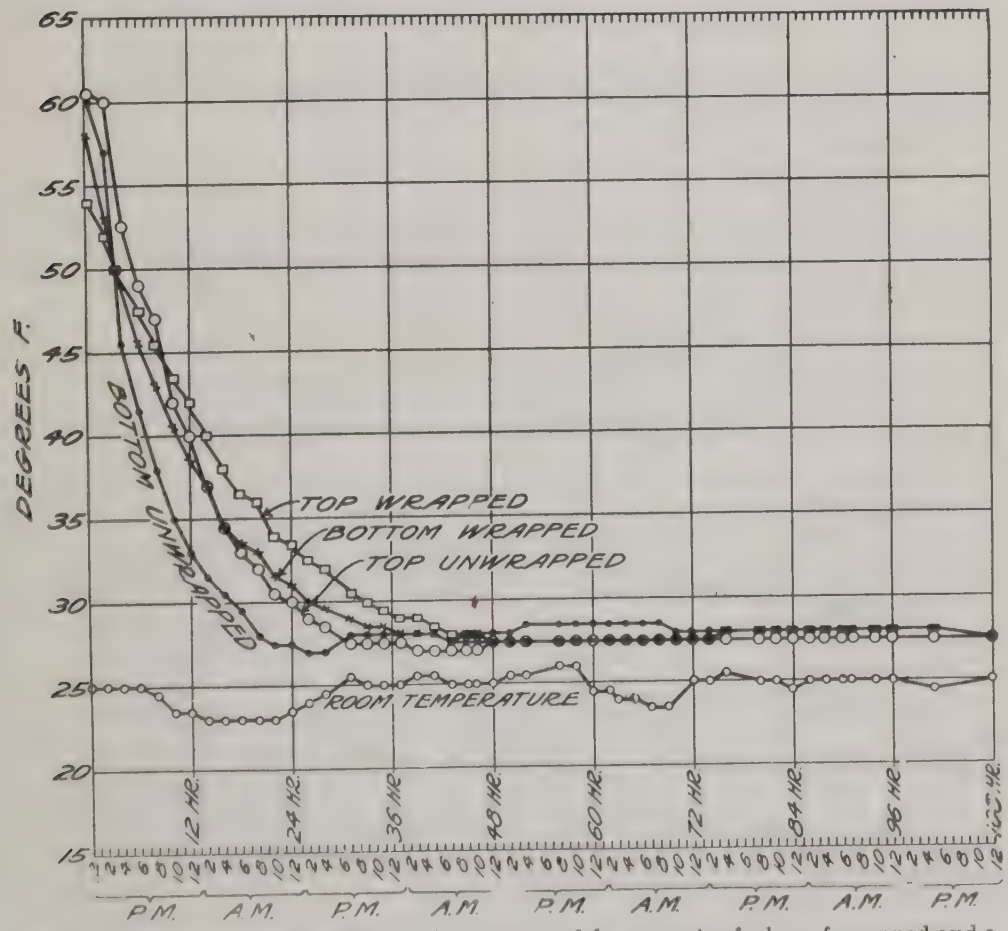

FIG. 7.—Temperatures obtaining in the upper and lower parts of a box of wrapped and a box of unwrapped York Imperial apples, during exposure to external temperatures around 25° F. for 120 hours

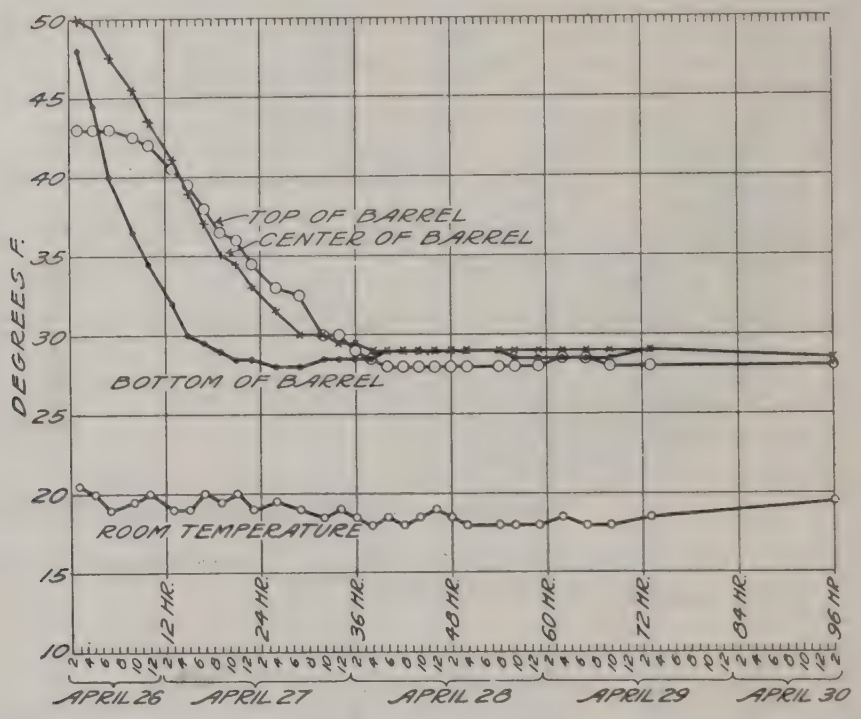

FIG. 8.—Temperatures obtaining in different parts of a barrel of unwrapped Winesap apples, during exposure to external temperatures at or below 20° F. for 96 hours

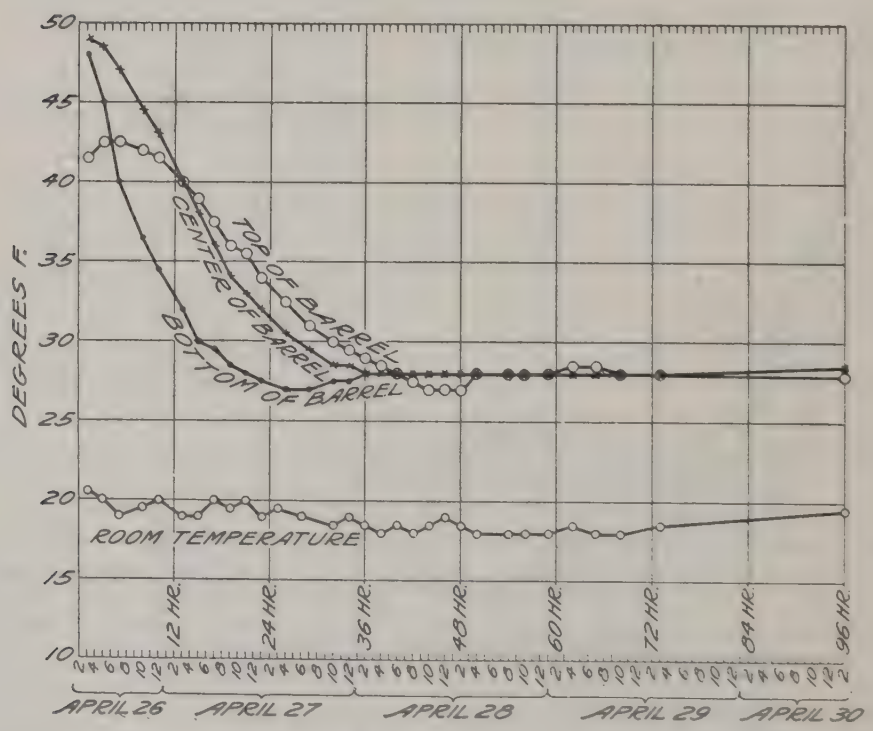

FIG. 9.—Temperatures obtaining in different parts of a barrel of unwrapped apples, during exposure to external temperatures at or below 20° F. for 96 hours

dents of packing, as mentioned above. After the first 16 hours the fruit in the top of the barrel is warmer than that elsewhere. Freezing began in the fruit at the bottom of the barrel after about 24 hours; in 12 more hours, or 36 hours from the beginning of the experiment, apples in the other positions also began to freeze.

For purposes of comparison the temperature curve for the bottoms of the two barrels of Winesap apples frozen at 20° F. and at 25° are presented together in Figure 10. Although the two initial temperatures are not the same, making exact comparison impossible, there is some indication of a more rapid rate of temperature fall in the bottom of the two boxes indicates that wrapping tends to equalize these differences, at least at the exterior temperatures prevailing during the experiment, and to make the temperature more uniform throughout the box. In the box of unwrapped apples freezing took place in amounts sufficient to influence the temperature curve not long after 12 hours. While there is some indication of freezing between 24 and 36 hours in the wrapped apples, the temperature curves do not assume a more permanent horizontal direction until 42 hours.

The relative lengths of time for which the temperature curves for the bottom of the boxes remain horizontal, or at the freezing point, should be noted.

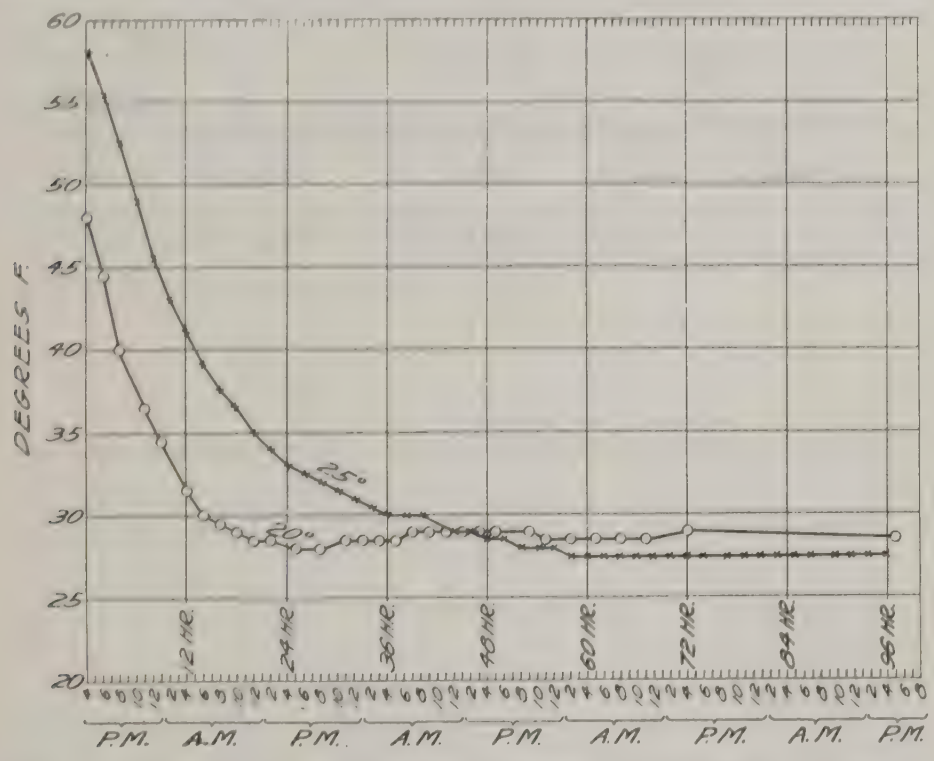

FIG. 10.—Comparison of the temperatures obtaining in the bottom of a barrel of unwrapped Winesap apples, during exposure to external temperatures around 25° F. for 96 hours with those obtaining in a barrel during exposure to temperatures at or below 20° F. for the same length of time

barrel exposed to 20° or below, while undercooling is definitely indicated in this case. The apples at the lower temperature began to freeze about 18 hours earlier.

The graphs showing the temperatures for the wrapped and unwrapped apples exposed to 20° F. or below are given in Figure 11, several facts being brought out in the curves. The much more rapid drop in temperature in the box of unwrapped apples is clearly defined, and both the top and bottom readings indicate that undercooling occurred, being particularly marked in the bottom. Consideration of the comparative differences existing between the readings for the top and bottom of the

At the close of the experiment the bottom temperature for the unwrapped apples was 3° lower than for the wrapped ones. That this has a practical bearing on the possible injury to the fruit will appear obvious in the light of other experiments in the temperatures at which freezing injury occurs and emphasizes the value of wrapping boxed fruit as a means of partial protection from low temperatures and resultant freezing.

Figure 12 compares the curves for the apple temperatures in the bottom of the barrel and in the lower part of the boxes. The comparative rates of the temperature drop in the barrel and box of wrapped apples clearly show

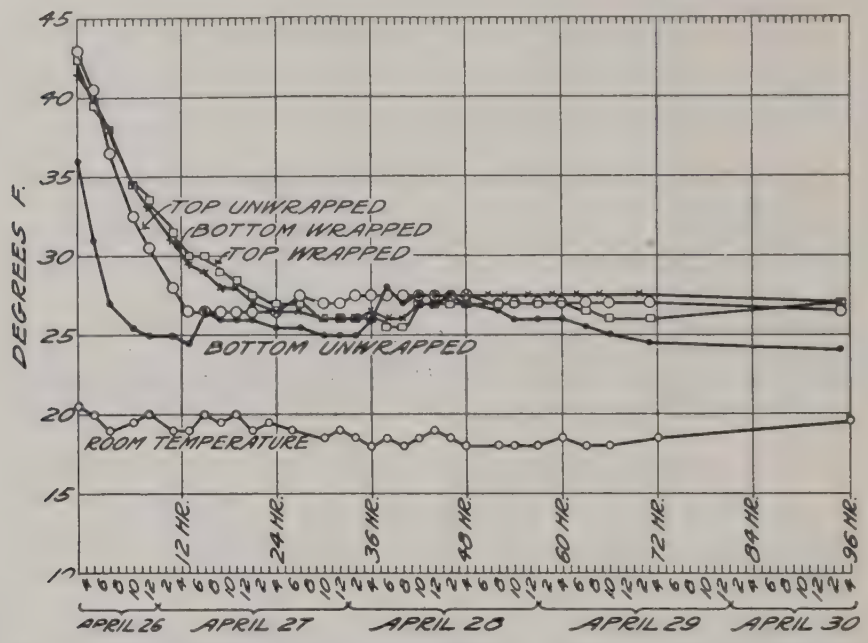

FIG. 11.—Temperatures obtaining in different parts of a box of wrapped and in a box of unwrapped Winesap apples, during exposure to external temperatures at or below 20° F. for 96 hours

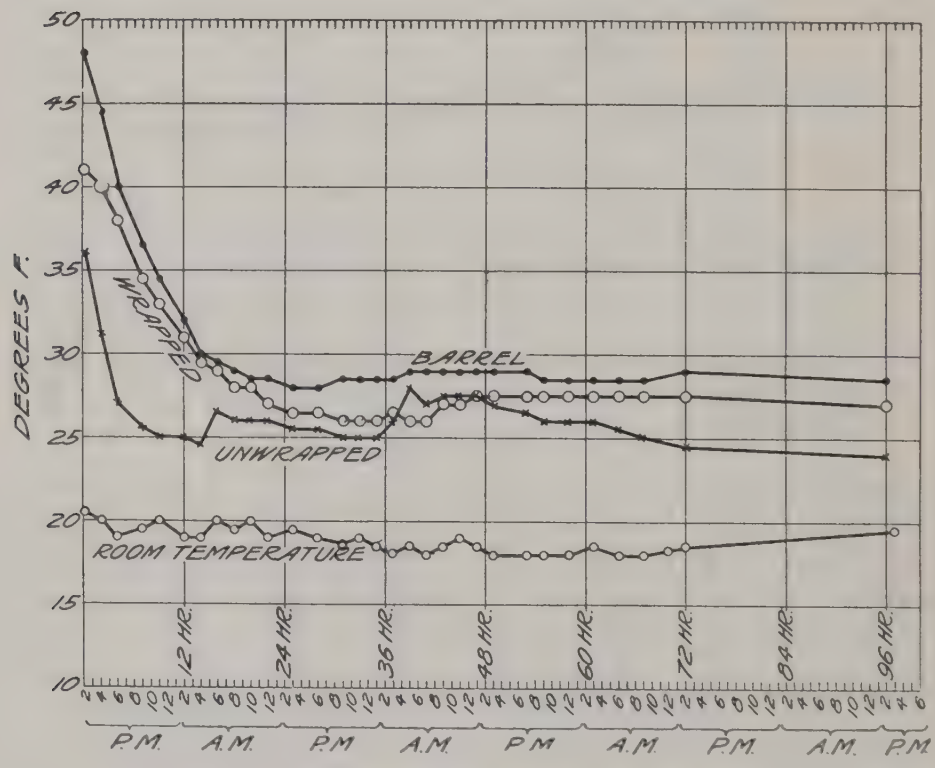

FIG. 12.—Comparison of the temperatures obtaining in the bottom of a box of wrapped, a box of unwrapped, and a barrel of unwrapped Winesap apples, during exposure to external temperatures at or below 20° F.

that the volume of fruit to be cooled tends to slow down the rate of cooling, and that wraps on the fruit produce a similar result. There is evidence that some of the fruit undercooled in both boxes and it is strikingly noticeable in the box of unwrapped fruit.

INJURY RESULTING FROM EXPOSURE OF APPLES IN PACKAGES TO FREEZING CONDITIONS FOR DIFFERENT PERIODS OF TIME

In addition to the lots described above in which the temperature change within the packages was observed, other lots were exposed to freezing conditions and removed at 24-hour intervals. After remaining at a temperature of 65° F. for 3 days the apples were examined for injury with the purpose of determining in 24-hour periods how long apples of different varieties in such packages could be exposed to certain freezing conditions without materially injuring the market value of the whole package.

Grimes Golden apples which had been in cold storage two weeks were exposed to temperatures from 19.5° F. to 21°, but about 20° most of the time. The fruit was packed in a 4 by 3 pack, 5 tiers high, in northwestern apple boxes with lids. York Imperial apples which had been in storage about three weeks were exposed to temperatures from 21° to 25°, but about 23° most of the time. Yellow Newtowns which had been in storage about five weeks were exposed to temperatures from 21° to 23.5°, but about 22° most of the time (Table IX).

The data shown in Table IX indicate that under prevailing conditions the Grimes Golden apples could hardly be exposed to a temperature of 20° F. for 48 hours without marked depreciation in market value. Undoubtedly 7.5 per cent of severe injury would be too high to be overlooked. The York Imperial apples showed only about 5 per cent severe injury after 48 hours, but the prevailing temperatures were somewhat higher. The Yellow Newtown showed about 5 per cent severe injury after a 72-hour exposure with a prevailing temperature slightly higher than that which obtained for the Grimes Golden, indicating that they were somewhat more resistant to freezing injury than the Grimes Golden.

The regular packing scheme used for each of these varieties permitted observations on the degree to which apples suffered visual freezing injury in different parts of the boxes. Apples in the interior lying in the second and third tiers from the top in any box seemed to be least affected by freezing conditions prevailing outside. Greater visual injury was found in apples touching the sides of the box, the corner apples being especially susceptible. If, however, the freezing conditions were severe as to either length or degree of exposure, the degree of visual injury found in the apples composing the lowest tier was generally so great throughout the tier that possible differences in injury intensity existing in apples at the side and at the center were not so apparent. The apples in the top tier in general showed somewhat more injury than the two immediately below it but considerably less than the two lowest tiers.

The varying power of resistance of different apples to conditions bringing about visual freezing injury was clearly demonstrated in these experiments. Specimens were found which showed very little injury after exposure to severe

TABLE IX.—*Percentage of visual injury in apples exposed for various periods of time at freezing temperatures and held at 65° F. for three days before inspection*

Average freezing-room temperature	Period of exposure	Percentage of visual injury			Total number of apples
		None	Trace	Severe	
GRIMES GOLDEN FROM VIRGINIA					
20° F	24 hours	100.0	0	0	122
	48 hours	82.8	9.7	7.5	124
	72 hours	67.5	13.0	19.5	123
YORK IMPERIAL FROM VIRGINIA					
23° F	48 hours	79.3	15.9	4.8	126
	72 hours	77.5	9.9	12.6	111
	96 hours	57.5	15.9	26.6	113
YELLOW NEWTON FROM VIRGINIA					
22° F	48 hours	98.8	1.2	0	87
	72 hours	89.7	5.7	4.6	88
	96 hours	70.4	17.1	12.5	88
	120 hours	52.0	14.0	34.0	100

freezing conditions, though surrounded on all sides by severely injured apples. Tiers of fruit often contained apples showing a wide range in amount of injury present in their tissues.

EFFECT OF STORAGE FOLLOWING FREEZING UPON THE AMOUNT OF VISUAL INJURY IN APPLES

Four lots of apples, Yellow Newtown, Winesap, Rome Beauty, and Delicious, were exposed to somewhat similar freezing conditions at different times. They had been stored at 32° F. in commercial apple boxes previous to the experiments described here. Storage following the exposure to freezing conditions took place in the temperature averaging about 21°. After exposure for definite periods, one crate was removed to 65° where it was held three days, while another was placed in 32° for a month. Even after six days' exposure the damage was not very serious in apples stored in 65°, while of those put in 32° for a month, even those held at 21° eight days showed only a moderate amount of severe damage, although this would probably have increased materially if the fruit had been kept warm several days after removal from cold storage.

The Rome Beauty apples had been in 32° F. storage nearly three months when they were exposed in slat crates

TABLE X.—*Percentage of visual injury in apples exposed for varying lengths of time to freezing temperatures, and subsequently held at 65° F. for three days, or at 32° for a month before inspection*

Average freezing-room temperature	Period of exposure	Subsequent storage	Percentage of visual injury			Total number of apples
			None	Trace	Severe	
YELLOW NEWTOWN FROM VIRGINIA						
21° F	24 hours	32° F	100.0	0	0	100
	48 hours		100.0	0	0	100
	72 hours		100.0	0	0	100
	96 hours		60.3	15.9	23.8	126
WINESAP FROM VIRGINIA						
21° F	48 hours	65° F	87.3	9.3	3.4	118
	96 hours		97.9	2.1	0	136
	144 hours		86.5	8.5	5.0	141
	192 hours		39.7	30.7	29.6	166
	48 hours	32° F	100.0	0	0	175
	96 hours		90.2	9.8	0	142
	144 hours		100.0	0	0	147
	192 hours		89.6	5.6	4.8	124
ROME BEAUTY FROM VIRGINIA						
22° F	72 hours	65° F	97.6	0	2.4	85
	96 hours		68.8	11.8	19.4	93
	144 hours		0	70.1	29.9	87
	192 hours		0	18.9	81.1	90
DELICIOUS FROM WASHINGTON						
22° F	72 hours	65° F	97.4	2.6	0	113
	120 hours		94.7	1.8	3.5	113
	168 hours		66.3	31.8	1.9	113

containers used during the exposure. The results obtained have been combined and are presented in Table X.

The Yellow Newtown apples, after five weeks in cold storage at 32° F., were exposed, in jumble pack in slat crates with no lid, to temperatures from 19° to 22.5°, but averaging 21°. After freezing, the fruit was held at a temperature of 32° for a month. The data given in Table X indicate that an exposure of 72 hours was not particularly damaging to the market value of the apples of this variety, but that 96 hours resulted in serious injury.

The Winesap apples, after about two months storage at 32° F., were exposed in slat crates without lids to a without lids to freezing room temperatures from 21.5° to 24° averaging about 22° most of the time. The subsequent storage was in 65° for three days. The data in Table X suggest that the apples suffered too severely after 72 hours' exposure to be in marketable condition in the original package.

The Delicious apples were exposed to the freezing temperatures in their original package but with wraps removed. The fruit had been in 32° F. storage about two months. The freezing-room temperatures ranged from 20.5° to 24°, averaging about 22° most of the time. Subsequent storage was in 65° for three days. Mealiness be

comes very pronounced in this variety after a certain amount of freezing, but discoloration does not appear so commonly (Table X).

It will be noted that there is some variation in the amount of injury to different varieties when they are exposed for the same periods of time to freezing temperatures such as prevailed during the experiment. The small amount of visual injury found in Delicious apples, as a rule, is an example of this variation. Hence, the periods of time for which apples in packages may be exposed without serious depreciation in immediate market value will differ not only with the variety, but with the changing factors in packing, handling, and other conditions which we are not yet able to recognize.

In general, it may be said that for temperatures such as prevailed during these tests, which averaged between 21° and 22° F., the exposure period must not be over 72 hours if serious damage is to be prevented and 48 hours is a much safer figure for some varieties. The fruit here discussed was not wrapped. As shown below, wrapping may considerably extend the period during which fruit may be exposed to low temperature without injury.

WRAPPING AS A RETARDANT OF FREEZING INJURY

Since it has been shown that wrapping materially retards the cooling in the fruit (see above, p. 110 to 111), it could be expected to reduce the amount of visual injury. Data are given in Table XI on the effect of exposing wrapped Yellow Newtown apples, packed in northwestern apple boxes, 4-3 packs, 5 tiers high, to temperatures from 21° to 23.5° F. for different lengths of time. Data are also given for Delicious apples, exposed in boxes as originally packed, to freezing-room temperatures from 20.5° to 24°, averaging 22° most of the time. Wrappers were removed from the latter apples in some boxes, but this packing arrangement was not otherwise disturbed. After exposure to these temperatures for the required time both groups of apples were held at 65° for three days before examination.

A comparison of results with wrapped and unwrapped lots, shown in Table XI, indicates that wrapping markedly retards visual injury. For instance, the percentage of injury for unwrapped Delicious apples exposed five days is not radically different from that for wrapped apples exposed nine days. There is a similar difference if 7-day wrapped fruit is compared with 12-day unwrapped fruit. These observations apply only to the amount of visual injury developed.

TABLE XI.—*The effect of wrapping fruit on the amount of visual injury in Yellow Newtown and Delicious apples after exposure to freezing temperatures averaging 22° F. for various lengths of time*

Period of exposure	Wrapped or unwrapped	Percentage of visual injury			Total number of apples
		None	Trace	Severe	
YELLOW NEWTOWN FROM VIRGINIA					
72 hours	Unwrapped	89.7	5.7	4.6	88
72 hours	Wrapped	96.6	3.4	0	88
120 hours	Unwrapped	52.0	14.0	34.0	100
120 hours	Wrapped	88.4	4.2	7.4	95
168 hours	Unwrapped	29.5	14.7	55.8	95
168 hours	Wrapped	72.1	9.7	18.2	93
DELICIOUS FROM WASHINGTON					
72 hours	Unwrapped	97.4	2.6	0	113
120 hours	do	94.7	1.8	3.5	113
168 hours	do	66.3	31.8	1.9	113
208 hours	Wrapped	89.4	10.6	0	113
288 hours	do	60.3	35.2	4.5	113
360 hours	do	0	80.5	19.5	113

Wrapping apples not only retards visual injury, but has other beneficial effects during thawing. It retards the rapid water loss which often takes place in the fruits, especially when thawing occurs at comparatively low humidities, and also lowers the amount of injury due to bruising while hard frozen, the latter feature being important if the fruit is hard frozen while in transit and subjected to jolting and jarring.

EFFECT OF WILTING ON FREEZING INJURY

Two collateral lots of Grimes Golden apples were picked early in the season, and one lot was subjected to freezing temperatures after only a short storage period at 32° F. The other lot was held in slat crates in storage at 32°

nearly four months before exposure to freezing conditions. The apples in this lot were badly wilted. Both lots were subjected to freezing temperatures which were never above 22.5° and were mostly about 21°.

That wilting retarded visual injury when apples were frozen is evident, especially in those lots exposed for the longer periods of time (Table XII).

THE EFFECT OF REPEATED FREEZING AND THAWING UPON THE FREEZING POINT AND AMOUNT OF VISUAL FREEZING INJURY

In order to study the effect of repeated freezing and thawing on freezing points of apples, also upon the amount of visual injury which results, thermocouples were used to obtain the internal temperatures, and when the apples had started to freeze they were removed and thawed by placing in a warm room over night. Each apple was then replaced on the thermocouple which had been used to read the first freezing point and another determination made. In this way the same thermocouple was used for all readings on any one apple.

The first of the series of experiments was made up of 24 Rome Beauty apples thawed at 70° F. after freezing. For the first time, the average freezing point was 28.8°, for which the freezing room temperature ranged from 21.7° to 22°. The second freezing on the same apples gave an average freezing point of 29.5° with a similar room temperature, the raising of the freezing point amounting to 0.8°. Another experiment with 23 Rome Beauty apples with freezing-room temperature from 21.7° to 22° as before, but for which the thawing temperature was 50°, gave 29.4° for the first and 29.6° for the second average freezing point. Five Delicious apples, with temperature near 22°, showed the first average freezing point to be 29.2°, the second 29.4°. Ten Yellow Newtown apples, frozen three times with temperature near 22°, showed the first average 28.3°, the second 28.7°, the third 28.7°. These figures indicate that the freezing points of apples are slightly higher after they are once frozen.

Rome Beauty apples in slat crates without lids were used in the experiments to determine the effect of repeated freezing and thawing on visual injury. The room temperature was between 21° F. and 22° most of the time, with a few intervals at 20°. The crates were held at 65° for three days between the intermittent freezing periods of 72 hours and those of 96 hours, and this was also true at the end of the periods of continuous exposures (Table XIII).

Apparently there is an increase in the amount of injury with each successive exposure to freezing conditions. As noted above, the freezing point is apparently higher with successive freezing and thawing, and it is probable that successive exposures to freezing conditions result in a greater total quantity of ice formed in the tissue with successive freezings following thawing. Comparison of the repeatedly frozen lots with those continually exposed to like low temperatures for similar periods shows that freezing injury is much more severe following 144 hours continuous exposure than following two exposures of 72 hours each, with thawing between. This again is probably associated with the amount of ice formed in the tissues, for the internal temperature of the fruit would more nearly approach the air temperature during a single long exposure than during two exposures of equal total length, but with an interval of high temperature between.

TABLE XII.—*Effect of wilting upon visual injury in Grimes Golden apples after freezing at temperatures averaging 21° F.*

Period of exposure	Condition of fruit	Percentage of visual injury			Total number of apples
		None	Trace	Severe	
48 hours	Unwilted	82.8	9.7	7.5	124
	Wilted	94.5	4.7	.8	127
72 hours	Unwilted	67.5	13.0	19.5	123
	Wilted	90.9	8.3	.8	133
96 hours	Unwilted	66.7	14.3	19.0	126
	Wilted	78.8	18.3	2.9	137
120 hours	Unwilted	32.5	23.0	44.5	126
	Wilted	67.5	20.5	12.0	125

TABLE XIII.—*Effect of repeated freezing at temperatures about 21° F. and of repeated thawing upon the amount of visual injury in Rome Beauty apples*

Number of times exposed	Period of each exposure	Percentage of visual injury			Total number of apples
		None	Trace	Severe	
	COMPARISON OF SUCCESSIVE INTERMITTENT PERIODS				
1	72 hours	97.6	0	2.4	85
2	72 hours	88.4	3.5	8.1	86
3	72 hours	0	88.7	11.3	71
1	96 hours	68.8	11.8	19.4	85
2	96 hours	0	56.5	43.5	85
3	96 hours	0	64.5	35.5	93
	COMPARISON OF INTERMITTENT PERIODS WITH CONTINUOUS EXPOSURE OF SAME HOUR TOTAL				
2	72 hours	88.4	3.5	8.1	86
1	144 hours	0	70.1	29.9	87
3	72 hours	0	88.7	11.3	71
1	216 hours	0	12.6	87.4	95
2	96 hours	0	56.5	43.5	85
1	192 hours	0	18.9	81.1	90
3	96 hours	0	64.5	35.5	93
1	288 hours	0	0	100.0	93

CHANGE IN THE FREEZING POINT OF APPLES HELD IN COLD STORAGE

The opinion has been expressed that the freezing point of apples held in cold storage is higher during the latter part of the storage period than at the time of placing in storage, and that at the end of the season the apples are more susceptible to visual freezing injury. While this injury in frozen fruit is more common as the storage season progresses, work with two varieties held at 32° F. throughout the season does not confirm the view that the freezing point thus rises. After apples are picked and placed in storage the tissue undergoes progressive physiological changes which eventually lead to maturity and overripeness, and possibly render the apple less resistant to freezing temperatures. This may account for the apparent greater visual injury in apples frozen toward the end of the storage season with the freezing point quite constant throughout. These physiological changes are also indicated by the more rapid development of mealiness under freezing conditions late in the storage season. But a progressive and sustained raising of the freezing point is not indicated (Table XIV).

TABLE XIV.—*Freezing points of Rome Beauty and Ben Davis apples held continuously in a temperature of 32° F. and tested at intervals of about a month through the storage season*

Date of test	Freezing point			Freezing-room temperature range		Number of apples used
	Average	Maximum	Minimum	Maximum	Minimum	
	ROME BEAUTY FROM VIRGINIA					
	°F.	°F.	°F.	°F.	°F.	
Nov. 2	29.1	29.8	28.4	21.4	21.1	42
Dec. 9	29.3	29.9	28.4	22.2	21.8	46
Jan. 10	29.3	29.8	28.6	23.4	23.2	47
Feb. 10	29.4	30.1	28.5	22.0	21.7	47
Mar. 13	29.1	29.9	28.7	23.5	23.1	21
	BEN DAVIS FROM VIRGINIA					
Nov. 7	29.0	29.4	28.7	22.2	20.3	24
Dec. 18	28.8	29.2	28.2	22.4	21.9	45
Jan. 15	29.0	29.5	28.3	22.2	21.9	42
Feb. 14	29.0	29.5	28.6	22.7	21.8	34
Mar. 13	29.0	29.5	28.4	23.5	23.1	23

EFFECT OF DIFFERENT THAWING TEMPERATURES ON FROZEN APPLES

Shippers and handlers of apples generally believe that frozen apples are injured less by gradual thawing, or storage at low temperatures, after freezing, than by rapid thawing at high temperatures. While evidence on this question is not altogether clear, the data in Table X indicate that somewhat more severe injury occurred in fruit thawed at 65° than in fruit thawed at 32°. Very rapid thawing

by immersing in warm water has been found to result in more severe injury than thawing even in 65° air. Consequently, thawing of commercially frozen fruit at moderately low temperatures is to be recommended. Aside from the question of actual freezing injury, the breakdown following freezing will go on much more rapidly at high temperatures. Unless the fruit is so severely frozen as to be unmarketable, handling at as low a temperature as possible will give most satisfactory results. But holding at low temperatures will not result in the recovery of apples so severely frozen as to be seriously discolored inside. Sudden transfer of frozen fruit to warm dry conditions may cause wilting, as the water resulting from melting ice crystals in the intercellular spaces of the tissue may not be reabsorbed by the cells if the thawing process is such as to induce a rapid exchange of water vapor to the outer air.

TABLE XV.—*Effect of freezing at temperatures from 20° F. to 21.5° on the softening of Yellow Newtown apples, as measured by the pressure tester, and on acidity and amount of visual injury found*

Period of exposure to freezing temperature	Period and temperature subsequent storage	Pressure in pounds necessary for puncture		Percentage acidity as malic acid	Number of apples showing visual injury		
		Pared	Unpared		None	Trace	Severe
None	None	16.5	22.3	0.653			
Do	24 hours at 65° F	15.6	22.0	.709			
7 hours	do	16.4	22.4	.695	16	6	3
17 hours	do	15.1	20.9	.599	11	6	8
24 hours	do	14.2	20.2		15	7	3
31 hours	do	14.1	21.6	.618	11	5	9
48 hours	do	13.5	19.3	.627	3	6	16
72 hours	do	12.0	19.7	.589	1	6	17
None	1 month at 32° F	15.4	22.8	.592			
7 hours	do	14.2	20.9		18	2	0
17 hours	do	14.0	19.6	.546	16	4	1
24 hours	do	13.1	19.6		16	2	2
31 hours	do	13.6	19.7	.563	14	5	1
48 hours	do	12.4	18.4	.545	10	8	2
72 hours	do	11.8	18.7		5	4	11

EFFECT OF FREEZING AS DETERMINED BY THE PRESSURE-TEST METHOD AND BY ACIDITY DETERMINATIONS

The work on visual freezing injury indicates an increase in severity and extent of injury as apples are subjected to progressively longer periods of exposure. To determine whether there is also a progressive change not evident to inspection by the usual method, experiments were carried out with a few varieties exposed to low temperatures for different periods of time. In addition to examination for visual injury the fruit was tested for softness by pressure according to the method already described; and acidity determinations were made on samples of apple tissue. These samples were obtained by cutting out plugs of tissue with a cork borer and pulverizing by passing through a sampling press of the type described by Clark (*2*); 100 gm. of tissue were weighed out, boiled, and made up to 1,000 cc. with distilled water. The samples were preserved with toluol, and after extraction for three days were filtered and titrated with phenolphthalein as indicator. The results are expressed in percentage of the wet weight of the tissues, the acid being calculated as malic.

In other experiments Yellow Newtown apples which had been held in storage at 32° F. about five months were subjected to temperatures from 20° to 21.5°. The fruit was exposed without packing or wrapping, arranged on a wooden platform so that each apple was several inches away from surrounding apples. At invervals sample lots of 20 to 24 apples were removed, one lot being placed in a temperature of 65° for 24 hours, the other in cold storage at 32° for one month, after which these lots were compared with unfrozen apples from the same general storage. Both the frozen and unfrozen fruit taken from the 32° storage were tested immediately (Table XV).

Ice had formed in the majority of the apples at the 17-hour period, and at all periods longer than 17 hours it may be assumed that the freezing process was adding to the amount of ice first formed. The data for the pressure tests show some variation especially in the column in which unpared apples are considered, but the figures for tests with pared apples seem to describe more accurately the condition of the interior tissue of the fruit, because tests with unpared

fruit necessarily include the resistance of the skin to rupture, which may possibly not be affected even in severe cases of freezing injury.

The data indicate a progressive softening in apples that have been frozen for increasingly longer periods of time, both when they were tested after 24 hours and when they had been in cold storage a month. Comparison of the pressure tests and the amount of visual injury show that appreciable softening can be detected at periods when visual injury is quite negligible in any particular lot. This indicates that apples are actually softened by the ice formation incident to freezing, and are softer and more mealy following thawing, even though no visual injury has occurred. This means that frozen apples are nearer to the end of their storage life. They can not be held so long as unfrozen fruit and be in good condition for merchandising after removal from storage.

The acidity changes show a distinct but irregular decrease with freezing in the case of the Yellow Newtown apples. This irregularity may be due to variations in the samples, for distinct variations occur even in unfrozen lots.

Another experiment was carried out with Ben Davis apples which had been held about five months in 32° F. They were subjected to freezing temperatures at about 22° for different periods of time. The procedure was the same as that employed in the experiments with Yellow Newtown apples discussed above, except that at each removal hour one sample lot was placed in 65° for a week (Table XVI).

The data are similar in character to those obtained for the Yellow Newtown apples, and show that as the periods during which the apples are frozen and held at freezing temperatures are lengthened, the fruit is softer upon thawing. It probably is also true that softening during subsequent storage is hastened by the freezing injury. It is noticeable that the visual injury and the pressure-test figures do not show increases of similar degree, and that softening occurs when visual injury is not present. The increase in acidity noted during storage is probably only apparent, and may result from a loss of water from the tissues, since the acidity is calculated to the wet weight of the tissues.

TABLE XVI.—*Effect of freezing at temperatures around 22° F. on the softening of Ben Davis apples in lots of 20, as measured by the pressure tester, and on acidity and amount of visual injury found*

Period of exposure to freezing temperatures	Period and temperature of subsequent storage	Pressure in pounds necessary for puncture		Percentage acidity as malic acid	Number of apples showing visual injury		
		Pared	Unpared		None	Trace	Severe
None	None	12.1	15.6	0.412			
Do	24 hours at 65° F	12.4	17.4	.396			
8 hours	do	11.1	15.5	.425	16	2	0
16 hours	do	11.5	15.5	.435	16	3	1
24 hours	do	9.7	13.7	.411	17	3	0
32 hours	do	11.1	14.6	.433	20	0	0
41 hours	do	10.7	14.6	.436	20	0	0
48 hours	do	10.7	15.3	.448	16	4	0
None	1 week at 65°	11.0	16.3	.349			
8 hours	do	11.1	15.4	.450	20	0	0
16 hours	do	10.8	14.3	.397	14	4	2
24 hours	do	10.7	14.6	.406	13	2	5
32 hours	do	10.3	14.0	.418	16	1	3
41 hours	do	9.8	13.7	.421	16	1	3
48 hours	do	8.5	12.6		10	3	7
None	1 month at 32°	10.5	15.3	.308			
8 hours	do	10.7	14.5	.407	20	0	0
16 hours	do	10.5	12.9	.377	14	3	3
24 hours	do	10.0	13.5	.375	12	3	4
32 hours	do	9.8	12.9	.356	16	0	4
41 hours	do	9.6	13.3		11	1	7
48 hours	do	9.1	11.7	.378	14	2	4

The above experiments were carried on at temperatures distinctly below the freezing points of the fruit used. In order to determine whether changes occur at temperatures just below the freezing point, tests were made on Winesap and Yellow Newtown apples grown in Virginia which had previously been in storage at 32° F. nearly six months. The two varieties were tested at the same time, with the freezing room held at temperatures ranging from 27° to 28°, except for a short period of 26° at the beginning. Fruit thermometers were inserted in scattered apples when they

were exposed to the low temperatures and were read every 24 hours. In this way an estimate of the temperature conditions in the whole lot was obtained. While this practice is not so satisfactory as readings at shorter intervals, the temperature changes in frozen apples while ice is being formed are rather slow at the freezing room temperatures used, so the method proved satisfactory (Table XVII).

of a thoroughly frozen apple. The acidity in a few instances shows a decrease from that found in unfrozen apples, but it is slight and irregular, and not significant.

Since the differences noted above are small, although distinct enough so that they seem noteworthy, another series of experiments was made on apples subjected to temperatures which remained between 26° F. and 26.5°.

TABLE XVIII.—*Effect of freezing, at a temperature just below their freezing point, on the softening of Winesap and Yellow Newtown apples, as measured by the pressure tester, and on the acidity and amount of visual injury found*

Periods of exposure to freezing temperatures	Period and temperature of subsequent storage	Pressure in pounds necessary for puncture		Percentage acidity as malic acid
		Pared	Unpared	
	WINESAP			
None	None	13.9	19.0	0.560
Do	24 hours at 65° F	13.2	17.6	.528
24 hours	do	13.6	18.5	.548
48 hours	do	12.8	17.4	.550
72 hours	do	13.1	16.9	.557
None	2½ weeks at 32°	13.6	17.7	.529
24 hours	do	13.2	17.2	
48 hours	do	12.6	17.5	.517
72 hours	do	12.7	17.6	.550
	YELLOW NEWTOWN			
None	None	16.2	23.4	0.570
Do	24 hours at 65°	15.7	22.3	.514
24 hours	do	15.9	23.6	.519
48 hours	do	14.8	22.6	.543
72 hours	do	14.4	21.3	.558
None	2½ weeks at 32°	13.8	21.6	.526
24 hours	do	13.6	21.2	
48 hours	do	13.2	21.0	.499
72 hours	do	13.6	21.3	.513

The average freezing point of the Winesap apples used in this experiment was 28° F. and of the Yellow Newtown apples 28.8°. The readings at the 72-hour period indicate that the apples were still at their freezing point at that time, or very near it, as they probably were also at the 48-hour period, though the amount of ice in the tissue had undoubtedly increased. Table XVIII presents the pressure test and acidity figures for sample lots of these apples withdrawn at 24-hour intervals when the temperatures were read. The storage and testing procedure was similar to that used in other experiments of the same general character. No visual injury was detected.

As in the experiments conducted at temperatures around 22° F., there is indication that the softening of apples is hastened by freezing. Although the differences are slight, the amount of ice formed in the apples held at a temperature just below the freezing point may be small, and none of the fruit was in the hard wrinkled condition

Five Virginia varieties, which had been held at 32° F. about seven months, were used. Acidity determinations were made only for the first

TABLE XVII.—*Internal temperatures of 5 Winesap and 5 Yellow Newtown apples exposed to a temperature just below their freezing point for different lengths of time*

Apple No.	24-hour period	48-hou period	72-hour period
	WINESAP		
	°F.	°F.	°F.
1	29.0	28.5	28.0
2	29.0	28.5	28.0
3	28.5	28.5	28.0
4	28.5	28.0	28.0
5	29.0	28.0	28.0
	YELLOW NEWTOWN		
1	29.5	28.5	28.5
2	29.0	29.0	28.5
3	29.0	29.0	29.0
4	29.5	29.5	28.5
5	29.0	29.5	28.0

two, and the procedure of the experiment was similar to that of those already described, with subsequent storage at 32° for two and one-half weeks for Winesap and Yellow Newtown (Table XIX).

TABLE XIX.—*Internal temperatures of certain Winesap, Yellow Newtown, Rhode Island Greening, Ben Davis, and Rome Beauty apples while exposed to temperatures between 25° F. and 26° for different lengths time* [a]

Apple No.	Internal temperatures of the fruit and length of period		
	24-hour period	48-hour period	72-hour period
WINESAP			
	° F.	° F.	° F.
1	27.0	28.0	27.5
2	26.5	27.5	27.0
3	25.0	26.5	26.5
YELLOW NEWTOWN			
1	27.0	26.0	28.5
2	28.5	28.0	27.5
3	27.0	26.5	27.5
RHODE ISLAND GREENING			
1	29.0	27.5	27.0
2	26.5	28.0	27.0
BEN DAVIS			
1	29.0	27.0	27.0
2	29.0	27.0	27.0
ROME BEAUTY			
1	27.5	28.5	28.0
2	27.0	29.0	27.5

[a] Average freezing points of the varieties used were: Winesap, 28°; Yellow Newtown, 28.8°; Rhode Island Greening, 28.8°; Ben Davis, 28.9°; Rome Beauty, 29°.

The temperatures indicated that ice formation had taken place in many apples at the 24-hour period; and the hard-frozen condition of the fruit at the 48-hour period showed that it had proceeded to a considerable extent in all of the fruit. A drop in the apple temperatures would indicate that the major portion of the ice possible in the tissue at that temperature had formed, and that the fruit was losing heat more rapidly to the environment than it was gaining from the heat of fusion as new amounts of ice were formed.

As has been observed in apples frozen at temperatures considerably below and just below their freezing point, the fruit of the five varieties subjected to temperatures near 26° F. showed a progressive softening as the time of exposure increased. In some varieties this is more noticeable than in others, due not to varietal peculiarities alone, but also to such conditions as differences in the size of the apples, the water content of the fruit, and their effect on the degree of ice formation.

TABLE XX.—*Effect of freezing apples at temperatures between 25° F. and 26° upon softening as measured by pressure tester*

Period of exposure to freezing temperatures	Period and temperature of subsequent storage	Pressure in pounds necessary for puncture	
		Pared	Unpared
WINESAP			
None	None	13.5	16.7
Do	24 hours at 70° F	13.0	17.2
24 hours	do	12.6	15.8
48 hours	do	12.6	16.3
72 hours	do	11.9	15.2
None	2½ weeks at 32	12.7	16.2
24 hours	do	12.1	16.3
48 hours	do	12.4	15.4
72 hours	do	12.4	14.8
YELLOW NEWTOWN			
None	None	14.3	21.7
Do	24 hours at 70°	14.1	21.4
24 hours	do	12.9	20.5
48 hours	do	12.9	19.9
72 hours	do	11.4	18.6
None	2½ weeks at 32°	13.7	20.6
24 hours	do	13.8	19.8
48 hours	do	12.5	19.0
72 hours	do	12.0	18.7
RHODE ISLAND GREENING			
None	None	11.7	13.9
Do	24 hours at 70°	11.7	13.8
24 hours	do	10.0	12.6
48 hours	do	9.6	12.0
72 hours	do	9.0	11.4
BEN DAVIS			
None	None	15.1	19.9
Do	24 hours at 70°	15.0	18.8
24 hours	do	13.9	18.5
48 hours	do	13.1	17.6
72 hours	do	13.3	17.6
ROME BEAUTY			
None	None	12.4	15.9
Do	24 hours at 70°	11.1	15.4
24 hours	do	11.4	14.8
48 hours	do	11.7	15.6
72 hours	do	10.9	14.5

The acidity changes are not given in table form, since they were not significantly different and resemble those for earlier freezing tests. The majority of cases exhibited no visual injury whatever, though a few showed a slight trace.

SUMMARY AND CONCLUSIONS

1. Freezing-point temperatures of a considerable number of important commercial varieties of apples have been determined. The average freezing point of all the varieties, both eastern and western grown, was 28.5° F., the maximum 29.4°, the minimum 27.8°.

2. Cooling apples below their freezing points without the formation of ice in the tissue does not cause any visual injury or perceptible softening of the fruit.

3. Undercooling of isolated apples can sometimes be carried to a point as low as 7 or 8 degrees below their freezing point without ice formation, provided the fruit is left undisturbed.

4. Inoculation or the beginning of ice formation in apples takes place rather slowly at times, and ice formation in the tissues does not spread so rapidly as in some other plant organisms that have been studied.

5. The bruising of apples while they are hard frozen results in a much more serious injury than does a similar pressure on unfrozen fruit. Consequently, any handling of frozen apples will usually result in more or less severe bruising injury.

6. The severity of the injury caused by bruising frozen apples is lessened as the amount of ice in the tissues decreases during thawing.

7. Bruises made on apples before they are frozen do not become more serious as to depth when the fruit is frozen, nor do they change materially in appearance.

8. There are no significant differences in the depth of bruises on hard-frozen fruit when subjected to different thawing temperatures.

9. Visual freezing injury may be classed under two heads, that appearing in the tissues of the apple, and that appearing on the surface, both being found in the same apple or occurring independently.

10. Visual freezing injury exhibits a great variety of forms and intensities which depend not only on the severity and duration of the prevailing freezing temperatures, but also on other factors, such as the physiological condition of the fruit.

11. The point to which the internal temperature of an apple must be reduced before visual injury occurs varies widely with the time of exposure to the temperature, with the variety, and with the individual apple. Under conditions of moderate thawing rates and rather short exposures to freezing conditions, it was found that the interiors of the apples usually had to reach a point at least 2° to 3° below the actual freezing temperatures after ice formation had started, before visual injury occurred. As the internal temperature dropped lower there was a rapid increase in the amount and severity of the injury, due probably to the increase in the quantity of ice formed in the fruit.

12. The data on the rate of cooling down of different packages at different freezing temperatures emphasize that apples can be exposed in such packages for considerable periods of time without the occurrence of any considerable actual freezing. There is some evidence that apples scattered through different parts of the package do freeze and that the heat produced by the ice crystallization in their tissues raises the temperature of the mass of fruit as a whole sufficiently to delay freezing in it. It does not seem possible to carry the undercooling of apples in packages to the degree to which it has been shown to occur in isolated, undisturbed apples, surrounded on all sides by cold air.

13. The fruit in the lower part of a package held under freezing conditions is exposed to a greater danger of freezing injury than that in any other portion of the package.

14. Wrapping serves to hinder the loss of heat from fruit under freezing conditions, thereby delaying the formation of ice in the fruit tissues. In this way the amount and severity of the freezing injury may be reduced, as regards both that determined visually and by means of other tests.

15. Repeated freezing and thawing of apples causes a progressive increase in the freezing injury, though not so great an increase as a prolonged continuous exposure. Thus two exposures of 90 hours each to freezing conditions will cause more injury than one exposure of 90 hours, but less than one exposure of 180 hours.

16. There is no evidence of progressive and sustained raising of the freezing point of apples held in 32° F. during the storage season. The greater susceptibility of apples to freezing injury as the storage season progresses lies apparently in a change in the physiological condition of the fruit and is not determined by a changing freezing point.

17. The temperatures at which apples are ordinarily thawed have only a slight effect upon the subsequent condition of the fruit. Yet very high temperatures and low humidities are not recommended because of the rapid water loss from the fruit under such conditions and the more rapid development of other injurious effects.

18. There is a distinct weakening in the keeping quality of apples when they are frozen, even when there is no visual evidence that freezing has occurred. The tissues of frozen apples are softer and more mealy after thawing and in poorer condition to stand storage and marketing, even though no direct discoloration has occurred. The degree of this softening again varies with the exposure and intensity of the freezing period.

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OILED PAPER AND OTHER OILED MATERIALS IN THE CONTROL OF SCALD ON BARREL APPLES[1]

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INTRODUCTION

Scald is one of the serious storage and market diseases of apples, and is particularly bad on the fruit that is stored in barrels. The barrel package is a tight one and its bulk of fruit relatively large, with the result that the apples cool more slowly than those in smaller and more open packages and also have less opportunity for the elimination of moisture, odors, or other waste products. In addition to this, a number of the varieties that are usually packed in barrels are naturally susceptible to scald and several of them are largely grown in the more southern orchard sections where picking and packing often begin while the weather is warm. These various conditions have made scald a particularly serious problem in the storage of barrel apples. The present paper gives a report of experiments in the use of oiled paper and other oiled materials for the control of scald in the barrel package.

HISTORICAL

Investigations looking to the control of apple scald extend over the last quarter of a century. They have developed a number of methods of reducing the disease and several of these have been put into practice to an extent to be of material value to the apple industry.

The first definite contribution in the study of scald was made in 1903 by Powell and Fulton (*16*).[2] They found that mature fruit scalded less than immature, and well-colored apples less than poorly colored ones, that less scald developed at a storage temperature of 31° to 32° F. than at a temperature of 36° F. or higher, and that delays in reaching storage were extremely favorable to the development of the disease.

Following this pioneer investigation no extended study of scald was made for a number of years. In 1904 (*4*) and again in 1909 (*5*) Beach published notes supporting the results of Powell and Fulton and in 1913 Greene (*12*) reported further supporting evidence. Greene found, however, that apples often scalded less in good cellar storage than in cold storage and that apples that were delayed in reaching storage sometimes scalded less than those that were stored immediately.

Ramsey, McKay, Markell, and Bird (*19*) in 1917 obtained results on the northwestern box apples similar to those of Powell and Fulton on the eastern barrel apples. Their investigations covered a period of four years and their experimental data gave conclusive proof that bad scald could be greatly decreased by picking the fruit at proper maturity. Apples that were delayed two weeks in reaching storage usually developed considerably more scald than those that were stored immediately and less scald developed in 32° F. storage than in 35° F. storage. They made withdrawals from storage at intervals of four to six weeks and reported data showing the progressive development of scald during the storage period.

Brooks and Cooley (*6*) (1917) and Brooks, Cooley, and Fisher (*7*) (*8*) (*9*) (*10*) (1919), (1923) found that immature apples scalded much worse than mature ones but that the mature ones might scald earlier in the storage season than the immature ones. Apples from heavily irrigated trees scalded worse than those from trees receiving more moderate irrigation. The rate of development of scald increased with a rise in temperature up to 15° or 20° C. (59° or 68° F.) but no scald occurred at 25° or 30° C. (77° or 86° F.). Scald was not due to the shock from the change of temperature on bringing the apples out of storage, but largely to the conditions that prevailed during storage. Delayed storage without ventilation resulted in increased scald but

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[2] Reference is made by number (italic) to "Literature cited," pp. 134–135.

delayed storage with free air movement over the apples served as a partial preventive for storage scald. Apples scalded less in boxes, baskets, hampers, and ventilated barrels than in the usual tight barrels. Increasing or decreasing the oxygen content of the storage air had little or no effect upon scald; increasing the carbon dioxide content tended to decrease scald. Excessive humidity favored the development of scald but was not usually an important determining factor in its development. Injuries apparently identical with scald could be produced by exposing apples to the vapors of esters and other substances that were constituents of the odorous products of the apple. Scald was reduced by coating the apples with a thin film of oil but the appearance of the fruit was often injured by the treatment. Oiled blotter material scattered through the package reduced the scald without injury to the fruit. Packing the apples in oiled wrappers (carrying 15 per cent or more of their weight in oil) was found to be the most satisfactory and efficient method of controlling scald. Ordinary unoiled wrappers were of little or no value in scald-control and paraffin wrappers far less efficient than the oiled ones. The value of the various oil treatments in scald-control was thought to depend largely upon the ability of the oils to absorb the odorous substances thrown off by the apples. The conditions leading up to scald were found to be of a cumulative nature, subject to remedial measures during the first six or eight weeks of storage but after that time often beyond remedy.

Power and Chesnut (*17*) (1920) found that the odorous constituents of apples consisted essentially of the amyl esters of formic, acetic, and caproic acids, with a very small amount of the caprylic ester and a considerable proportion of acetaldehyde. In a later publication (*18*) (1922) geraniol was added to the list of odorous constituents.

Whitehouse (*20*) (1919) stored Iowa apples under different humidities and found that slightly more scald developed with a relative humidity of 80 to 90 than with a relative humidity of 60 to 70. The degree of temperature had a much greater influence in the development of scald than the degree of humidity, a constant temperature of 32° F. giving the best results. Wrapping apples delayed the appearance of scald and paraffin paper retarded the disease slightly more than the ordinary wrapper.

A later report (*11*) (1922) from Iowa Agricultural Experiment Station stated that the use of oiled wrappers practically controlled scald in all cases tested.

Magness and Burroughs (*13*) (1922) pointed out the relation of wilting to scald resistance. They obtained more scald with apples exposed to a high humidity than with those exposed to a lower humidity, and were of the opinion that the effect of ventilation or air circulation in reducing scald might be mainly an action of drying of the surface of the fruit.

Marble (*14*) (*15*) (1922), (1923) also emphasized the relation of wilting to scald resistance. He thought that low temperatures had a depressing effect on apples and lessened the resistance of certain varieties to scald. Ventilation was effective in preventing scald if the temperature was high enough for the variety.

Adam (*1*) (1923) reported that Australian apples scalded more when immature than when mature. He found in agreement with others that scald was worse the higher the temperature, and that efficient ventilation practically eliminated the disease.

Baker (*2*) (*3*) (1924) reported results confirming the efficiency of the oiled wrappers in scald control. In an experiment on Grimes apples he found that shredded paper gave practically as good scald control as the wrappers.

EXPERIMENTAL RESULTS

The commercial results of the past two years seem to indicate that the oiled wrapper has largely solved the scald problem so far as box apples are concerned but it does not meet the requirements of the barrel packer. Experiments extending over a period of six years have proved that the oiled wrappers will largely control scald as it occurs in the barrel package and some growers have found it profitable to pack their apples in this manner, but the difficulty of placing wrapped apples in barrels and the cost of the operation make the oiled wrapper impracticable so far as the general barrel trade is concerned. The situation has created an increased demand for a method of scald-control adapted to the barrel package. The first method that suggested itself for controlling scald apart from the wrapper was that of applying the oil directly to the skin of the apples. A large number of tests of this sort were made and various mixtures of oils and waxes were tested. The results (*10*) were not satisfactory. In nearly

all cases the oils and waxes reduced the scald and in some instances almost entirely controlled the disease, but the apples had a greasy appearance upon removal from storage, the natural bloom was lacking, the skin was often abnormally green and in some cases it was definitely injured, turning brown much as if affected with scald.

Incidental results in various oiled wrapper experiments indicated that it ought to be possible to control scald without coating the skin with oil or actually inclosing the apple in a wrapper. In many of the oiled wrapper experiments only a part of the apples of the package were wrapped and this gave a chance to note the effect of the wrapper upon the adjacent unwrapped fruit. It was found that scald on the immediately adjacent apples was reduced to about one-fourth of that on the control barrels and that the scald on apples two or more layers distant from the oiled wrappers was reduced to about 60 per cent of that on the control. The results led to efforts to carry the oil of the package by other means than the wrapper or the direct application to the skin of the apple.

OILED BARRELS AND OILED LINERS

An experiment was made in which the barrels were soaked in oil. Approximately one pound of mineral oil was applied to the inner surface of each barrel and was absorbed by the wood. The effect that this treatment had upon the development of scald on Grimes Golden apples is shown in Table I.

Experiments were also made in lining the barrels with a double layer of ordinary desk blotters which had been saturated with oil. Approximately one pound of oil was used in each barrel. The tests were carried out on Grimes Golden, York Imperial, and Arkansas (Mammoth Black Twig). The results are shown in Table I.

The oiled barrels reduced the scald on the outside apples but had practically no effect upon those in the center of the barrel. The oiled liners gave similar results on the adjacent fruit and in some instances seemed to reduce scald upon the apples that were at a distance of several layers. Neither of the treatments was considered a practical success.

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OILED LAYERS

The effect of layers of oiled paper was also tested. A single layer of apples was placed in the barrel, then a layer of oiled wrappers, followed by another layer of apples, and this in turn by a layer of oiled wrappers. As many wrappers were used in a layer or paper as there were apples in the layer of fruit immediately beneath, thus making the quantity of paper in the barrel the same as if the apples had been wrapped and giving each apple two areas of contact with the wrappers. The experiment was carried out on two separate lots of York Imperial apples and the results are shown in Table II. The layers of oiled paper gave almost as good scald control as was obtained on the apples that were wrapped individually in similar paper.

As a commercial proposition there would obviously be an advantage in having circles of paper the size of the barrel rather than the wrappers but the above method furnished a means of testing whether a given amount of paper would have the same efficiency in scald-control when placed in layers between the apples as it would have if wrapped around the apples. The results seem to indicate that the actual inclosing of the apple in the wrapper is not a matter of extreme importance, at least for barrel fruit.

OILED BLOTTER STRIPS

Experiments were made in which ordinary desk blotters were heavily oiled, cut into strips ¾ by 6 inches, and scattered through the package. Approximately 3 pounds of oiled blotter carrying 1.5 pounds of oil were used in each barrel. The material was coarse and only fairly well distributed. The test was made on Grimes Golden, Delicious, Arkansas, and York Imperial apples. The results are given in Table I. Two or more barrels were included in each treatment of each variety.

The apples that were packed with the blotter strips had less than half as much scald as the untreated fruit, but several times more scald than the apples in oiled wrappers. The treatment was not considered a success from the practical standpoint.

TABLE I.—*The effect of oiled barrels, oiled liners, and oiled blotter strips upon the development of scald, 1919 experiments*[a]

Variety, locality, and storage period	Percentage of scald						
		Oiled barrels		Lined with blotters			
	Untreated barrels	Apples next to barrel	Apples in center of barrel	Apples next to blotters	Apples in center of barrel	Blotter strips	Oiled wrappers
Grimes Golden, Rockville, Md., Sept. 4, 1919, to Dec. 31, 1919	80	35	67	25	73	25	0
Delicious, Winchester, Va., Sept. 25, 1919, to Feb. 2, 1920	63	----	----	----	----	45	18
York Imperial, Leesburg, Va., Nov. 1, 1919, to Jan. 17, 1920	74	----	----	----	----	12	4
York Imperial, Rockville, Md., Sept. 26, 1919, to Jan. 15, 1920	70	----	----	21	48	11	0
Arkansas, Winchester, Va., Oct. 7, 1919, to Jan. 10, 1920	55	----	----	8	20	32	1

[a] The records were taken three days after the apples were removed from storage. During the after-storage period the fruit was held at a temperature of approximately 70° F. The percentages show the general severity of the disease, allowance being made for the surface area scalded, and the intensity of the scalds as well as the number of apples affected.

TABLE II.—*The effect of oiled straw, oiled paper, and oiled wrappers upon the development of apple scald, 1923 experiments*[a]

Variety, locality, storage period, and the nature of the oil treatment	Percentage of apples showing—			Degree of scald
	Bad scald	Slight scald	No scald	
Grimes Golden, Rockville, Md., Sept. 13, 1923, to Jan. 15, 1924:				*Per cent*
Untreated	73	25	2	87
Chopped oiled paper, 1 pound per barrel	4	20	76	8
Oiled wrappers	0	23	77	5
York Imperial, Vienna, Va., Oct. 11, 1923, to April 3, 1924:				
Untreated	70	21	9	71
Layers of oiled paper (wrappers)	6	7	87	4
Chopped oiled paper, 1½ pounds per barrel	2	17	81	5
Ribboned oiled paper, 1½ pounds per barrel	11	18	71	9
Chopped straw, unoiled, 4½ pounds per barrel	31	15	54	33
Chopped straw carrying ½ pound of oil; 5¾ pounds of oiled straw per barrel	0	20	80	4
Chopped straw carrying 1 pound of oil; 6 pounds of oiled straw per barrel	1	9	90	2
Oiled wrappers	5	25	70	6
York Imperial, Rockville, Md., Oct. 18, 1923, to Feb. 29, 1924:				
Untreated	94	6	0	76
Layers of oiled paper (wrappers)	7	27	66	8
Chopped oiled paper, 1½ pounds per barrel	2	31	67	7
Chopped oiled paper, 2¼ pounds per barrel	0	22	78	1
Ribboned oiled paper, 1 pound per barrel	0	18	82	1
Ribboned oiled paper, 1½ pounds per barrel	1	26	73	3
Ribboned oiled paper, 2¼ pounds per barrel	0	4	96	0.2
Oiled wrappers	4	19	77	5
Arkansas (Mammoth Black Twig), Inwood, W. Va., Oct. 29, 1923, to March 17, 1924:				
Untreated	79	21	0	89
Ribboned oiled paper, 1 pound per barrel	32	39	29	23
Ribboned oiled paper, 1½ pounds per barrel	0	29	71	6
Ribboned oiled paper, 2¼ pounds per barrel	8	12	80	9
Oiled wrappers	29	30	41	19
Ben Davis, Inwood, W. Va., Oct. 29, 1923, to May 20, 1924:				
Untreated	45	37	18	35
Ribboned oiled paper, 1½ pounds per barrel	0	0	100	0
Oiled wrappers	0	0	100	0

[a] An apple was counted as having slight scald when it had but a slight touch of brown that probably would have little if any effect upon its market value. When worse than this it was counted as having bad sleald. The percentages in the last column give the general severity of the disease in one set of figures alowance being made for the surface area scalded and the intensity of the scald, as well as the number of, apples affected.

OILED STRAW

In 1923, experiments were conducted with oiled wheat straw. The straw was cut into lengths of 1 or 2 inches, sprayed with oil and allowed to stand a week or more before using. It seemed quite oily immediately after the spraying but in a few days the oil was so completely absorbed by the straw that it was scarcely noticeable, especially in the cases of the lighter applications. One-half pound to one pound of oil was used with the straw in each barrel of apples. The test was carried out on two separate lots of York Imperial and the results are reported in Table II.

The straw punctured some of the apples and caused a slight increase in the number of rots. The dust from the straw was inclined to stick to the apples after removal from storage and detracted from their appearance. The straw itself, without the oil, seemed to have some value in scald control, reducing the disease to about half the amount found on the untreated barrels. The oiled straw gave practically complete scald control, making even a better showing than the oiled wrappers. It should be noted, however, that a larger quantity of oiled material was used in the case of the straw than with the wrappers.

SHREDDED OILED PAPER

In 1923 several of the companies that were manufacturing oiled wrappers also prepared some form of shredded oiled paper. Two different brands of this paper were tested in the experiments reported in Table II. The chopped paper had been cut into pieces about 1 inch wide and 1 to 2 inches long. It was well oiled and tightly pressed together, making it difficult to get the mass of paper in a finely divided condition suitable for thorough distribution in the package. The ribboned paper consisted of strips about one-fourth inch wide and 10 to 12 inches long. It was well oiled but loose and springy and seemed to enmesh the apples more thoroughly than the chopped paper. It also gave a nice appearance to the package. Both brands of paper were thoroughly and evenly distributed through the barrel. The experiment was conducted on Grimes Golden, Arkansas, Ben Davis, Yellow Newtown, and on two separate lots of York Imperial apples. Two or more barrels were included in each treatment of each variety. The results are reported in Table II.

The weight of oiled wrappers that would be required for wrapping a barrel of apples would vary with the size of the apples and the size and quality of the wrappers but would probably average about 1½ pounds. Where 1½ pounds of shredded oiled paper were evenly distributed through the barrel the average scald control was almost identical with that secured with the oiled wrappers and with the Arkansas variety it was distinctly better than with the wrappers. With 1 pound of shredded oiled paper to the barrel the results on Grimes Golden and Arkansas were poorer than those with the oiled wrappers, but the 1-pound treatment gave very satisfactory results on York Imperial. With 2¼ pounds of shredded paper to the barrel the results on the Arkansas were no better than with 1½ pounds, but with the York Imperial the 2¼ pounds gave better scald control than any other treatment. In general, the results indicate that shredded oiled paper, well distributed in the barrel, will give practically as good scald control as an equal weight of oiled paper used as wrappers.

DISCUSSION

The foregoing experiments give some additional information in regard to the nature of apple scald and furnish practical suggestions for the control of the disease in the barrel package.

The oiled barrels and the oiled liners were a failure although 1 pound of oil was used to the barrel. Three pounds of oiled blotter strips carrying 1.5 pounds of oil to the barrel gave very poor scald control. Five to six pounds of oiled straw carrying one-half to 1 pound of oil gave good scald control. Shredded oil paper and layers of oiled wrappers, used at the rate of 1.5 pounds to the barrel and carrying about 5 ounces of oil to the barrel in each case, gave practically the same scald control as was obtained by wrapping each apple in an oiled wrapper. The quantity of paper and oil per barrel was approximately the same with the layers and the shredded paper as where each apple was wrapped separately. The results indicate that it is not essential to scald control, at least in the barrel package, to have the apples actually inclosed in wrappers or to have the oiled material in contact with the apples at every point. On the other hand, the failure of the oiled barrels, the oiled liners, and the oiled blotter strips with their heavy applications of oil, indicates that the oiled material must be in close proximity to

the apples and that the distribution and the surface exposure of the material are even more important than the amount of oil carried.

Of all the treatments tested, the shredded oiled paper offers the greatest practical possibilities for the barrel package. The layers of oiled wrappers gave almost as good scald control, but the treatment as carried out in the experiments would not be adapted to practical operations. The apples were packed in definite layers, thus giving a more uniform contact between the wrappers and fruit than would be likely to be obtained under the customary methods of packing. The careful placing of the apples and the wrappers as packed in the experiment took almost as much time and labor as would have been required to actually wrap the fruit. The use of a larger and thicker sheet instead of the individual oiled wrappers would mean a saving in labor, but there would be less surface exposure and the additional strength and stiffness in the paper would result in less contact between the paper and the apples and would also probably mean a more open pack that might later become slack in storage. The method could hardly be used with satisfactory results without considerable care to place the apples in layers.

The experimental results indicate that under average conditions 1½ pounds of chopped or ribboned oiled paper to the barrel will give fairly satisfactory scald control provided it is well distributed in the package. Various methods have been suggested for securing this distribution under commercial packing-house conditions. One of the great obstacles in securing satisfactory results is the prevailing custom of running in too large quantities of apples at one time. If the apples were added a peck at a time and each addition followed by a sprinkling of oiled paper it should be readily possible to secure a good distribution of the material. This method would necessarily make some delay in packing, but if the apples were added in smaller quantity and the barrel shaken after each addition the final result should be a tighter pack with less necessity for crushing the apples at the tail of the barrel and probably a saving of the cost of plugging the packages later in the season. The most practical method of applying the oiled paper will of course vary with orchard and packing-house conditions.

SUMMARY

Various oiled materials have been tested for the control of scald in the barrel package.

Oiled barrels and oiled liners have reduced scald on the outside apples but have had little effect upon the package as a whole.

Oiled straw, shredded oiled paper, and layers of oiled wrappers have given practically as good scald control as wrapping the apples in oiled paper.

The success of these various treatments has apparently depended greatly upon the thoroughness with which the oiled material has been distributed in the package.

The shredded oiled paper furnishes the most promising method of scald control for the barrel package.

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THE GREENHOUSE LEAF-TYER, PHLYCTAENIA RUBIGALIS (GUENÉE)[1]

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INTRODUCTION

The larvae of a small moth known as the greenhouse leaf-tyer,[2] *Phlyctaenia rubigalis* (Guenée),[3] may be classed among the more important and destructive enemies of chrysanthemum, cineraria, snapdragon, and a long list of other ornamental and greenhouse plants. According to recent authoritative reports received by the Bureau of Entomology, this pest has, in several instances, occasioned complete destruction of the plants attacked. The need for more complete information regarding its life history and control under greenhouse conditions, together with the frequent complaints and repeated requests for more effective means of control, prompted the authors to make the present study of this insect as a greenhouse pest.

NATURE OF INJURY

The injury results almost exclusively from the feeding done by the larvae. Since the length of the larval period is slightly more than the combined egg and pupal stages, ample time is provided for them to become exceedingly troublesome and cause considerable damage. Feeding is normally restricted to the lower surface of the leaves, although on some host plants, as cineraria and chrysanthemum, the larvae have been observed to attack the upper surface and the bloom as well. At first the larvae skeletonize the foliage by eating small holes in the underside of the leaves so that only the upper epidermis remains intact. The injured areas which become pitted later coalesce and present a silvery appearance. As they grow older the larvae may devour the entire leaf tissue. As their common name implies, they spin a slight silken web, or tie two contiguous leaves together to form a shelter within which they remain while feeding. They develop voracious appetites, and when present in considerable numbers cause severe injury to the plants attacked, not only disfiguring them, but actually destroying the leaf surface to such an extent that the plants die (Pl. 1, A, B). When the food supply becomes exhausted they crawl away to other plants, and since they are able to feed on so many different varieties the infestation is spread very rapidly throughout the houses.

ECONOMIC IMPORTANCE

Although an apparently well-established pest in this country, the leaf-tyer intermittently causes such severe injury on certain greenhouse crops, especially chrysanthemums and cinerarias, that it demands prompt attention when a house has become infested. Considered primarily as a greenhouse pest, the citations below give some idea of the seriousness and the extent of damage which it may inflict. These include only a few of the more recent complaints and appeals for assistance which have been received by the Bureau of Entomology, although numerous early records are on file which might be mentioned further to emphasize the potentialities of this insect.

In 1909 it was reported from Portland, Oreg., as causing serious injury to violets in greenhouses, and in the same year a correspondent from Adrian, Mich., reported the larvae as being "present by millions on chrysanthemums, and devouring the plants so that it was impossible to secure cuttings." In 1912 a report from Huntsville, Ala., indicated that "two houses of chrysanthemums were stripped of their leaves, which only two weeks before had shown promise of a fair crop." In 1919 the senior author visited a florist in Vincennes, Ind., and

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[2] Sometimes referred to as "the parsnip webworm," "the chrysanthemum leaf-skeletonizer," and "the celery borer."

[3] Order Lepidoptera, family Pyralidae, subfamily Pyraustinae.

saw the destruction which this pest is capable of causing. In this instance the entire crop of chrysanthemums in two houses was a complete loss. During 1921 the leaf-tyers were reported as common in greenhouses throughout the Middle West and at Flint, Mich., where they caused severe injury to cinerarias.

FOOD PLANTS

Among the preferred hosts the following flowering and ornamental plants most susceptible to their attacks may be listed: Common chrysanthemum (*Chrysanthemum hortorum*), cineraria (*Senecio cruentus*), violet (*Viola tricolor*), rose (*Rosa* spp.), carnation (*Dianthus caryophyllus*), calendula (pot-marigold) (*Calendula officinalis*), sweet pea (*Lathyrus odoratus*), marguerite (*Chrysanthemum frutescens*), geranium (*Pelargonium hortorum*), snapdragon (*Antirrhinum* spp.).

In addition, they are more or less injurious to primrose (*Primula* spp.), ageratum (*Ageratum houstonianum*), marigold (*Tagetes* spp.), heliotrope (*Heliotropium peruvianum*), common petunia (*Petunia hybrida*), begonia (*Begonia* spp.), canna (*Canna indica*), dahlia (*Dahlia rosea*), wallflower (*Cheiranthus* sp.), nasturtium (*Tropaeolum* spp.), abutilon (*Abutilon* spp.), cyclamen (*Cyclamen persicum*), anemone (*Anemone japonica*), sage (*Salvia officinalis*), moonflower (*Calonyction aculeatum*), azalea (*Azalea* spp.), sultan snapweed (*Impatiens sultana*), spiderwort (*Tradescantia fluminensis*), blessed thistle (*Cnicus benedictus*), kenilworth-ivy (*Linaria cymbalaria*), ivy groundsel (*Senecio mikanioides*), ground-ivy (*Nepeta hederacea*), passion flower (*Passiflora caerulea*), feverfew camomile (*Matricaria parthenoides*), plumbago (*Plumbago capensis*), redspray ruellia (*Ruellia amoena*), (*Tydaea*) *Isoloma ocellatum*, lobelia (*Lobelia erinus*), speedwell (*Veronica* spp.), common lantana (*Lantana camara*), slender deutzia (*Deutzia gracilis*), goatsrue senna-pea (*Swainsona galegifolia*), China aster (*Callistephus chinensis*), coleus (*Coleus blumei*), fuchsia (*Fuchsia speciosa* [*hybrida*]), *Justicia furcata*, canary broom (*Cytisus canariensis*).

DISTRIBUTION

The greenhouse leaf-tyer is widely distributed throughout the United States, Canada, Central America, and South America, and is definitely reported as occurring in the District of Columbia and in the following States: Alabama, California, Colorado, Connecticut, Florida, Illinois, Indiana, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Mississippi, Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, Oregon, Pennsylvania, Texas, Vermont, Virginia, West Virginia, and Wisconsin.

SYNONYMY

Since 1854, when this American species was originally described by Guenée (*8, p. 398*)[4] as *Scopula rubigalis*, it has frequently been confused with the closely related European species known as *Phlyctaenia ferrugalis* Hübner-*Botys oblunalis*, described by Lederer (*10, p. 469*), and *Botis harveyana*, described by Grote (*7, p. 104–105*) in 1877, were regarded by Hampson as synonymous with the American species. In 1899 Hampson (*9, p. 242, 243*) catalogued *Pionea rubigalis* and *Pionea ferrugalis* as distinct species. Two years later both Slingerland (*13, p. 159*) and Chittenden (*3, p. 7*) used the name *Phlyctaenia rubigalis* in describing the greenhouse species. In 1902, however, Fernald (*in* Dyar, *5, p. 387*) reduced all these species to synonymy with Hübner's *ferrugalis*, a classification which has since been followed in economic literature down to the present time. Barnes and McDunnough in 1917 (*1, p. 133*) also regarded the two species as identical. The specimens used in these experiments have been identified by Messrs. Busck and Heinrich as *Phlyctaenia rubigalis* Guenée.

The following are the more important references to *Phlyctaenia rubigalis*, and its synonyms:

Scopula rubigalis Guenée, 1854, Spec. Gen. 8: 398.

Botys oblunalis Lederer, 1863, Wien. Ent. Monatschr., 7: 372, 469.

Botis harveyana Grote, 1877, Canad. Ent., 9: 104–105.

Pionea rubigalis Hampson, 1899, Proc. Zool. Soc. London f. 1899, p. 242.

Phlyctaenia rubigalis Chittenden, 1901, U. S. Dept. Agr., Div. Ent. Bul. 27, p. 7; Slingerland, 1901, N. Y. Cornell Agr. Exp. Sta. Bul. 190, p. 159.

Phlyctaenia ferrugalis Fernald, 1902, not Hübner, *in* Dyar's List N. Amer. Lep., p. 387; Barnes and McDunnough, 1917, not Hübner, Check List Lep. Bor. Amer., p. 133.

[4] Reference is made by number (italic) to "Literature cited" p. 158.

The Greenhouse Leaf-Tyer Plate 1

A.—House of chrysanthemums showing plants uninjured and in normal condition

B.—House of chrysanthemums showing plants severely injured by the greenhouse leaf-tyer

SYSTEMATIC DESCRIPTION OF PHLYCTAENIA RUBIGALIS GUENEE

For a number of years this American species has appeared in our lists and literature under the name *Phlyctaenia ferrugalis* Hübner, on the supposition that it was identical with the European species of that name.

On this supposition Fernald (*in* Dyar, *5, p. 387*) wrongly listed *ferrugalis* as an American species and placed *rubigalis* as one of its synonyms, although Chittenden (*3, p. 7–26*), previously had pointed out certain differences in the two species. He, as well as Slingerland (*13, p. 159–164*), correctly referred to the American species as *rubigalis* Guenée, following Hampson's (*9, p. 242*) determination.

A comparison of the genitalia of American and European specimens at once proved them two different species. *Phlyctaenia rubigalis* occurs throughout the United States, Central America, and South America. As far as we know *P. ferrugalis* does not occur anywhere on this hemisphere. All specimens which we have seen under this name from North and South America are true *rubigalis*.

The two species can be distinguished on several characters. Although the moths are very similar in size and color and difficult to separate with certainty without examining the genitalia, the outer transverse line on the forewing has a slightly different course, being more angulate on the costal edge than in *ferrugalis* and more outwardly curved on its dorsal half. The faint, oblique, dusky shade on the outer fourth of the forewing in *ferrugalis* is hardly present, or at least very faint, in *rubigalis*, and the marginal black dots are more distinct in *rubigalis* than in the European species.

An examination of either the male or female genitalia at once proves the distinctiveness of the two species. In *rubigalis* the tegumen of the male genitalia is narrower and more elongated than that of *ferrugalis* which is more abruptly shouldered. The differences in the anellus are very striking. In *ferrugalis* it is a simple, nearly rectangular plate, with two upper corners triangularly produced and with the incision between these broad and shallow; while in *rubigalis* it is a much more ornate structure, with the two upper triangular projections much longer and the interval between them correspondingly narrower and deeper, and with two branched and strongly scobinated lateral arms. (Compare fig. 1, A and B.)

The harps of *ferrugalis* are somewhat narrower than those of *rubigalis* and contain a cluster of very short, stout spines on the sacculus, represented in *rubigalis* by an ill-defined and more diffused patch of fine hairs; the clasper is stouter in *ferrugalis* than in *rubigalis* and penis contains only one strong, straight cornutal spine, while in *rubigalis* there are two, one of which is curved. Other minor differences on the male genitalia are shown in Figure 1, A and B.

In the female of *ferrugalis* the signum of the bursa copulatrix is considerably broader in proportion to its length than that of *rubigalis* and the chitinization around the ductus bursae and the genital opening is broader and more extended. (For differences in detail compare fig. 2, C and D.)

A single superficial color difference at once separates the larvae; in the European species there are two distinct and conspicuous black spots on each side of the thoracic shield, shaped like a crude exclamation point, while in *rubigalis* there is only a single elongate spot. In occasional specimens this may be partly broken up into two, but on the whole the character holds.

A detailed description of the various stages of *Phlyctaenia rubigalis* follows.

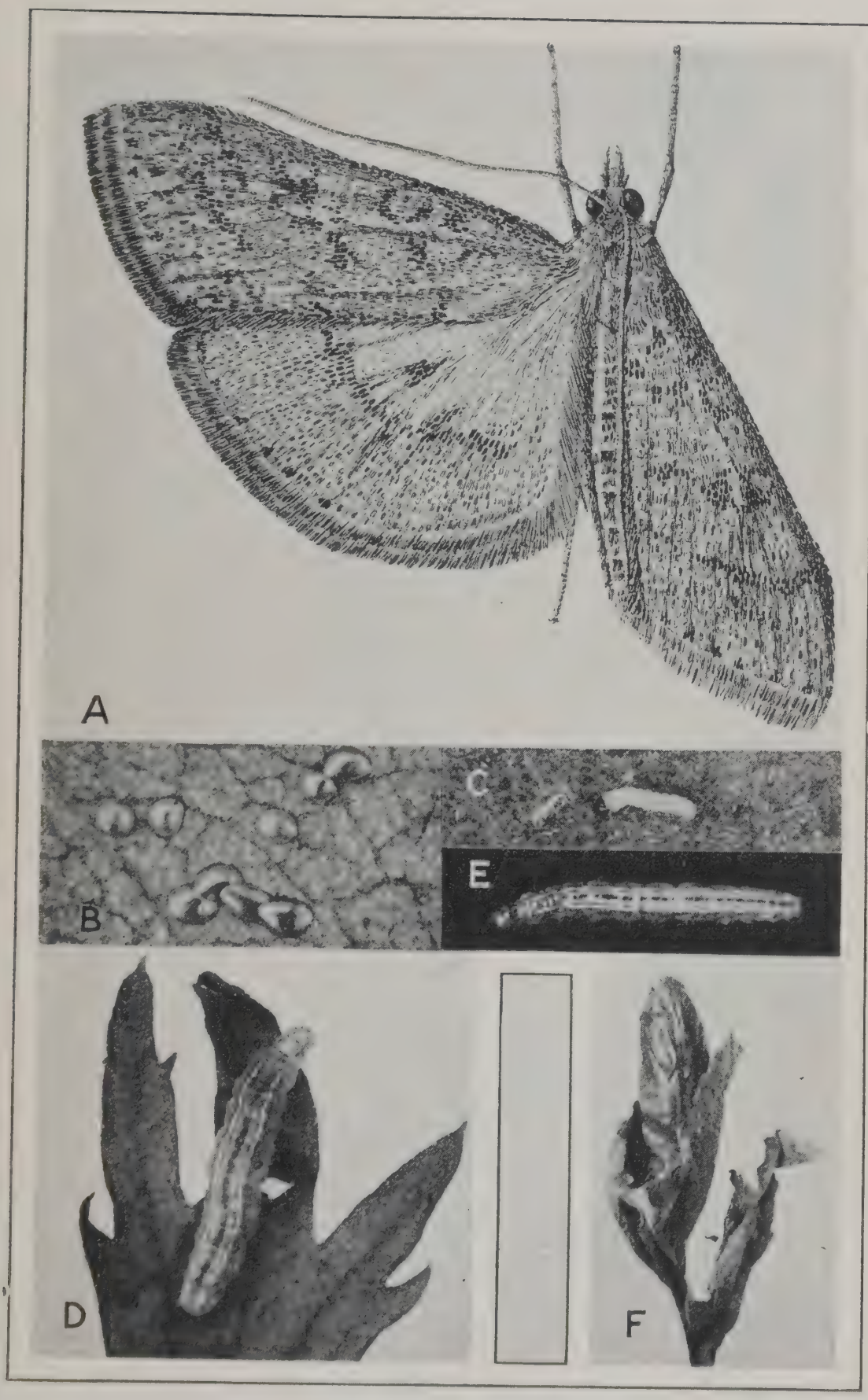
EGG

Egg 0.8 mm. long by 0.6 mm. broad, ovate, flattened, shiny and iridescent, whitish when newly laid, translucent, surface finely and irregularly reticulated.

The eggs are generally laid on the underside of a leaf, singly or in groups of from 2 to 12 or more, overlapping one another (Pl. 2, B).

LARVA

The larva (Pl. 2, C, D, E) is slender, cylindrical, tapering toward both extremities; without secondary hair; body pale green with a narrow, darker



The Greenhouse Leaf-Tyer

Plate 2

Phlyctaenia rubigalis: A.—Moth. B.—Egg clusters on portion of rose leaf. C.—Newly hatched larva. D.—Full-grown larva on marguerite leaf. E.—Full-grown larva. F.—Rolled marguerite leaf torn to expose pupa within. (All greatly enlarged)

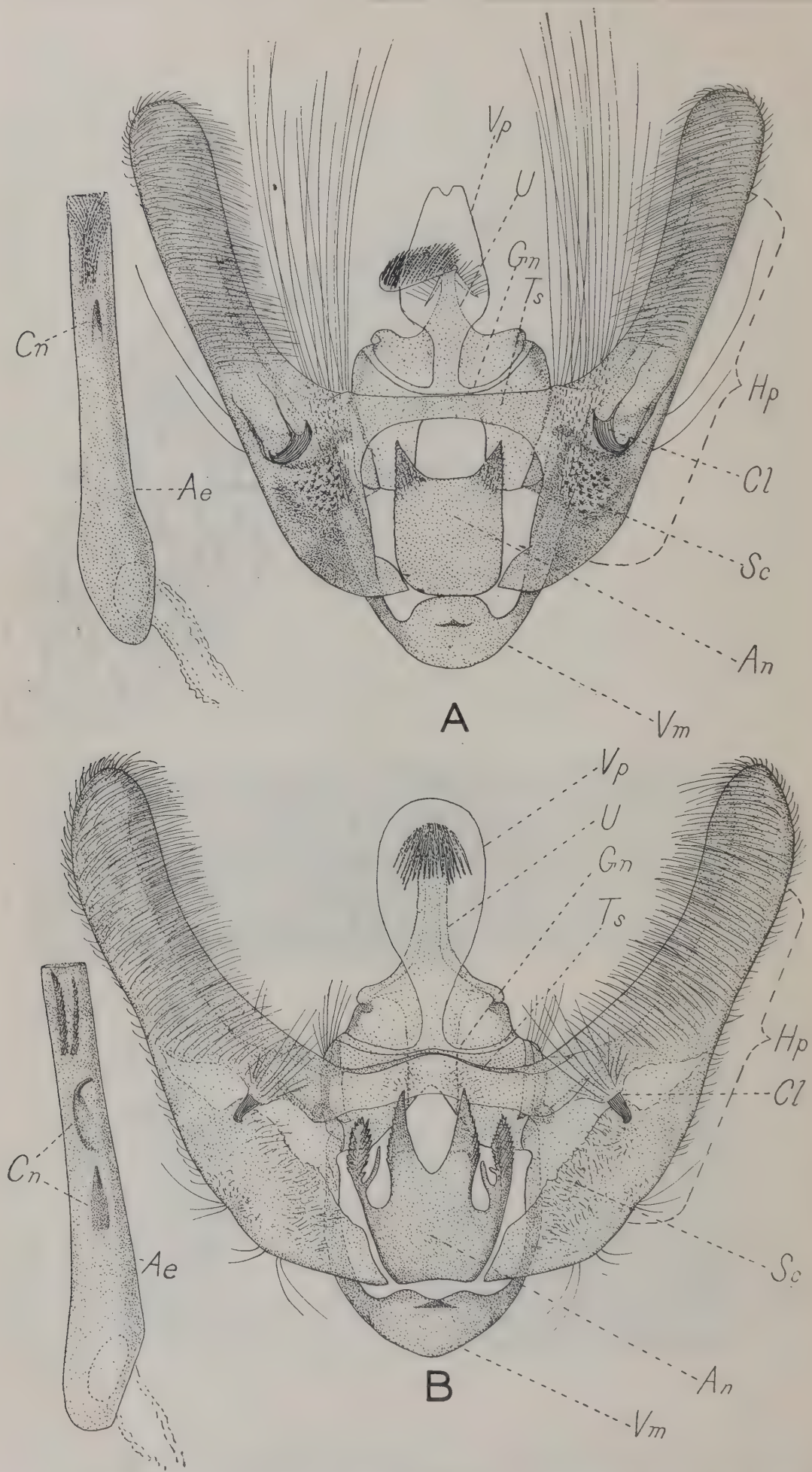


FIG. 1.—A, Male genitalia of *Phlyctaenia ferrugalis*, with detached aedoeagus at left; B, the same of *P. rubigalis*. *Ae*, Aedoeagus; *An*, anellus; *Cl*, clasper; *Cn*, cornuti; *Gn*, gnathos; *Hp*, harp; *Sc*, sacculus; *Ts*, transtilla; *U*, uncus; *Vm*, vinculum; *Vp*, ventral plate

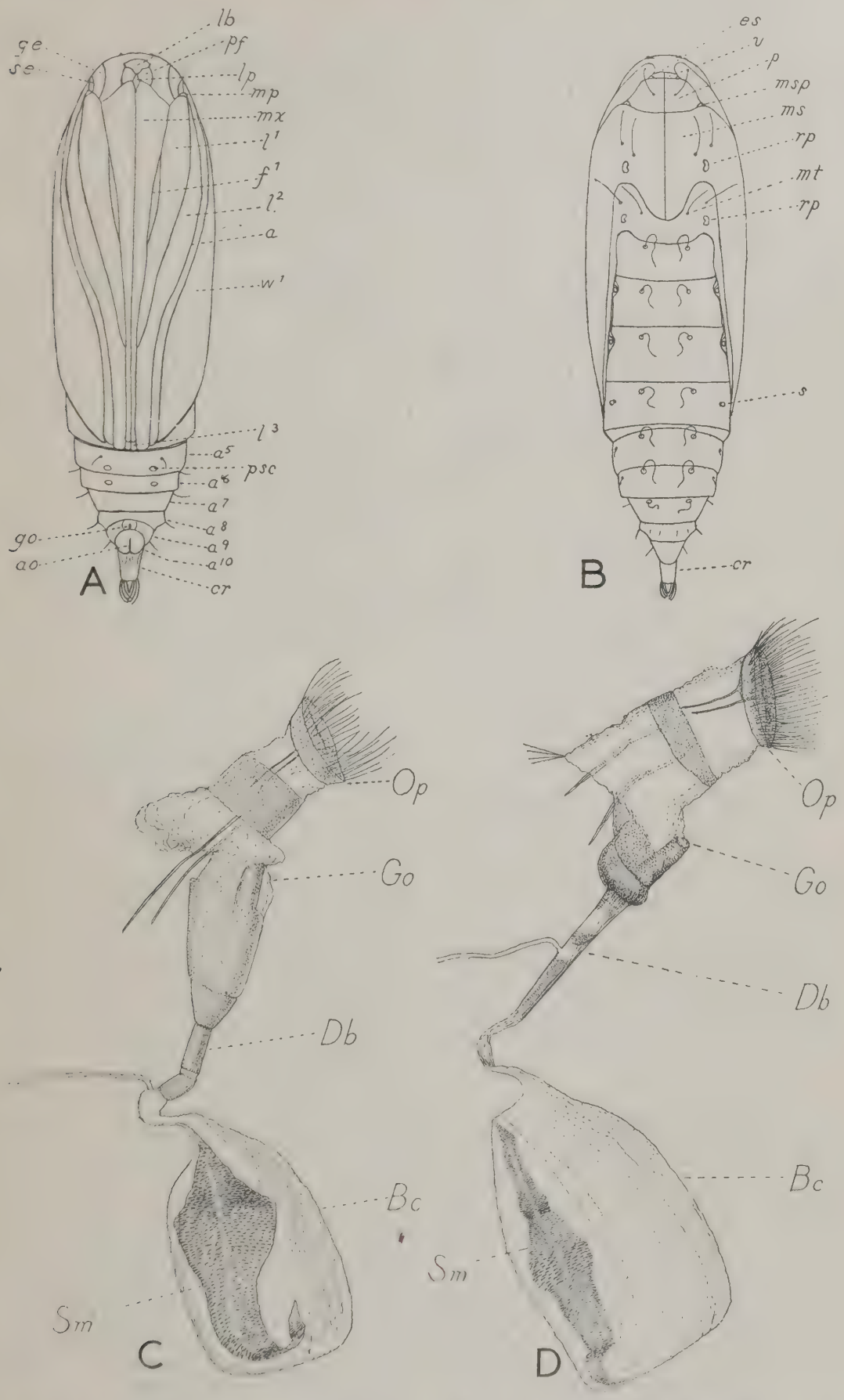


FIG. 2.—A, Pupa of *Phlyctaenia rubigalis*, ventral view; B, pupa of *P. rubigalis*, dorsal view; C, female genitalia of *P. ferrugalis*; D, female genitalia of *P. rubigalis*. Explanation of symbols applied to genitalia: *Bc*, Bursa copulatrix; *Db*, ductus bursae; *Go*, genital opening; *Op*, ovipositor; *Sm*, signum. Explanation of symbols applied to pupa: *a*, Antenna; *ao*, anal opening; *cr*, cremaster; *es*, epicranial suture; f^1, femur of prothoracic leg, *ge*, glazed eyepiece; *go*, genital opening; *lb*, labrum; l^1, prothoracic leg; l^2, mesothoracic leg; l^3, metathoracic leg; *lp*, labial palpi; *mp*, maxillary palpus; *ms*, mesothorax; *msp*, mesothoracic spiracle; *mt*, metathorax; *mx*, maxilla; *p*, prothorax; *pf*, pilifer, *psc*, proleg scar; *rp*, reniform pit; *S*, spiracle; *se*, sculptured eyepiece; *v*, vertex, w^1, forewing; a^5–a^{10}, abdominal segments 5 to 10.

green, dorsal longitudinal band, bordered on each side by a broader whitish band; under side of body yellowish (or greenish yellow), tracheae white showing through the skin. Thoracic legs slender and rather long; pale greenish yellow with two small, round, black spots on the outer side of the tibiae. Prolegs (fig. 3, D) slender and long stemmed; crochets (36–42) triordinal, in a penellipse. Anal fork absent. Tubercles and anal and thoracic shields of the body color; a single, conspicuous oval black spot on each side of the thoracic shield, lying back of seta 1b. Spiracles small, round, but very faintly pigmented; on 8th abdominal segment more than twice the size of the other abdominal spiracles and noticeably larger than those on prothorax. Skin smooth, except for the primary setae.

Body setae (fig. 3, E) pale yellow, moderately long. Prothorax *with only two setae on prespiracular shield* (4 and 5); II^a a trifle higher than I^a; II^b markedly higher than I^b, nearer to II^a than II^a is to I^a; I^b as near (or nearer to) I^c as I^c is to II^c, remote from I^a; only two punctures distinguishable (y and z), puncture y dorsocaudad of I^a. Mesothorax and metathorax with VI unisetose. Abdominal segments 1 to 8 with III above the spiracle; III^a not distinguishable; IV and V approximate and on a single chitinization beneath the spiracle; seta I longer than II, on abdominal segments 1 to 5 somewhat higher than II, but on 6th, 7th, and 8th abdominal segments on about the same level, absent on segment 9 of abdomen; group VII trisetose on proleg-bearing segments, bisetose on abdominal 7, and unisetose on abdominal 8 and 9; 9th abdominal segment with IV and V united in a single hair closely approximate to III; seta VI present and very long.

Head small; pale whitish or greenish yellow, faintly mottled with brownish yellow. Trophi very slightly pigmented. Ocellar pigment defined as small black spots under each ocellus.

Head capsule (fig. 3, A, B) somewhat flattened; ovate-orbicular in outline viewed from above; as wide as long; greatest width slightly before middle of head; incision of dorsal hind margin about one-fourth the width of the head (or a trifle less); distance between dorsal extremities of hind margin less than one-half the width of the head. Frons broad; longer than broad; reaching beyond middle of head. Adfrontal sutures extending to incision of dorsal hind margin. Longitudinal ridge short; considerably less than one-third the length of frons. Ocelli six; lenses well defined. Epistoma normal. Frontal punctures well separated; slightly forward of frontal setae; only distinguishable in cleared and prepared mounts of the head; distance from frontal seta F^1 to first adfrontal seta (Adf^1) greater than between Adf^1 and Adf^2; approximate to beginning of longitudinal ridge; puncture Adf^a very faint, somewhat nearer to Adf^2 than to Adf^1.

Epicranial punctures, seta G^1, and setae of ultraposterior group not distinguishable. Anterior setae (A^1, A^2, A^3) forming a very obtuse angle lying almost in a line with L^1. Posterior setae (P^1, P^2) slightly behind middle of head; P^1 well behind the level of Adf^1 and a trifle behind that of L^1; P^2 well behind the level of place of juncture of adfrontal ridges.

Ocellar setae (O^1, O^2, O^3) well separated; O^1 ventrad of ocelli 2 and 3, approximate to ocellus 3; O^2 ventrad of ocellus 1; O^3 anteroventrad of O^2, remote, farther from O^2 than from O^1. Subocellar setae triangularly grouped.

Length of full-grown larva 17–19 mm.

PUPA

The pupa (fig. 2, A, B; Pl. 2, F) is smoky brown with intersegmented areas of abdomen (especially from abdominal segments 5 to 7) pale yellow; smooth, except for a slight rugosity on dorsum of 7th segment of abdomen and some rather prominent dorsal setae; mesothorax and metathorax with a distinct, prominent, rather large, chitinized reniform pit on dorsum on each side of middle; wings extending to ventro-caudal margin of fourth abdominal segment; cephalic end bluntly rounded, tapering from mesothorax, vertex distinct, small; labrum, pilifers, and maxillary palpi well developed; labial palpi very small; prothoracic and mesothoracic legs not extending cephalad between sculptured eyepiece and antennae; maxillae broad and long, extending nearly the length of the wings, having only tips of metathoracic legs exposed; femora of prothoracic legs clearly exposed; prothoracic legs extending more than half the wing length; mesothoracic and metathoracic legs and antennae extending to tips of wings; proleg scars clearly visible on abdominal segments 5 and 6; mesothoracic spiracle discernible, but small and narrow, not prominently chitinized; abdominal spiracle distinct, not appreciably produced, small and round; anal and genital openings slitlike in both sexes; anal rise very slight, unarmed; cremaster present, prominent, moderately long,

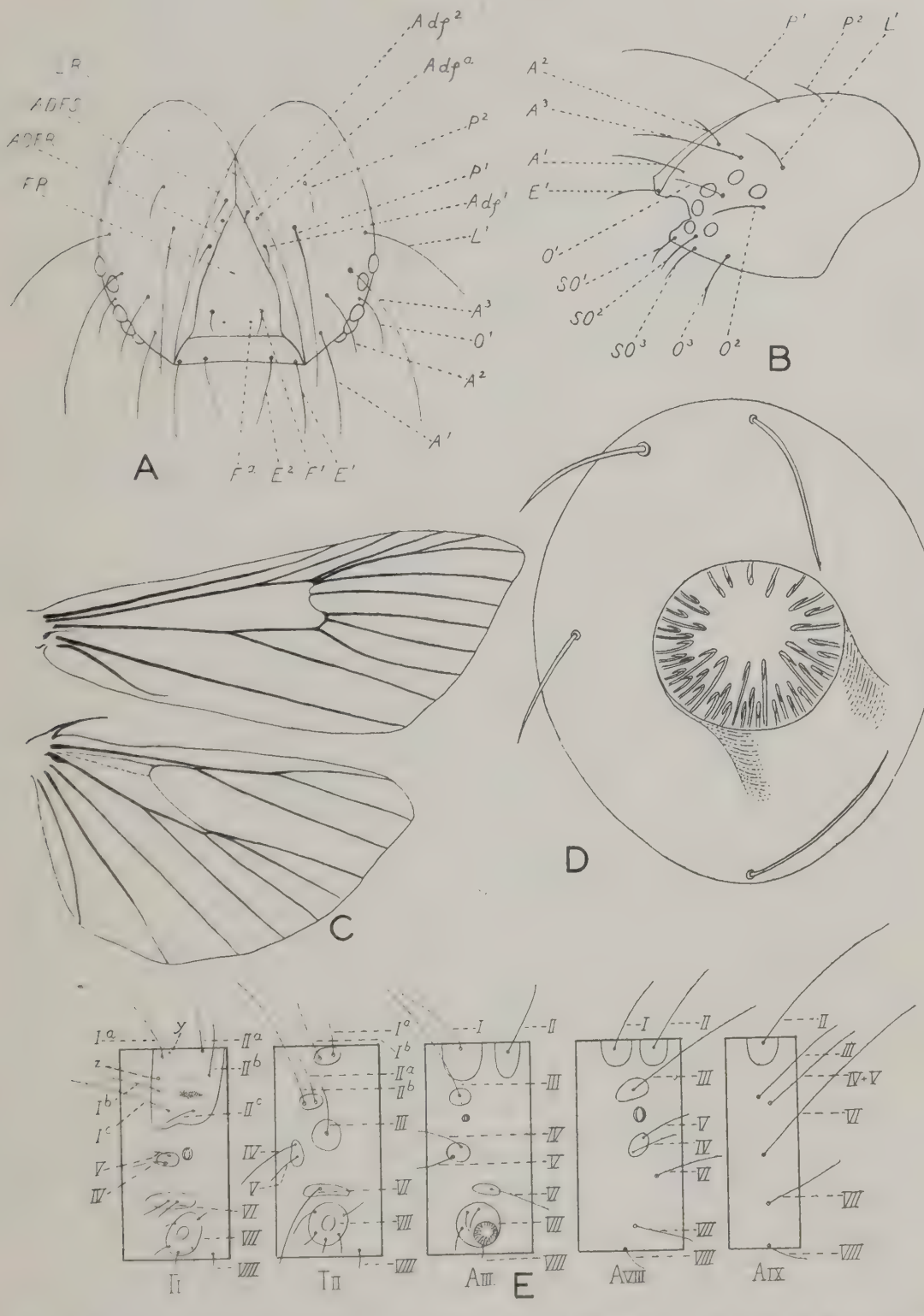


FIG. 3.—A, Head capsule of larva of *Phlyctaenia rubigalis*, front view; B, same, side view; C, Wing venation of same; D, Abdominal proleg of larva; E, Map of body setae of larva. Explanation of symbols: A^1, A^2, A^3, setae of anterior group of epicranium; Adf^1, Adf^2, Adf^a, adfrontal setae and puncture of epicranium; *ADFR*, adfrontal ridge of larval head; *ADFS*, adfrontal suture of larval head; E^1, E^2, epistomal setae; F^1, F^a, adfrontal setae and puncture of epicranium; *FR*, frons of epicranium; L^1, lateral seta group of epicranium; *LR*, longitudinal ridge of epicranium; O^1, O^2, O^3, setae of ocellar group of epicranium; P^1, P^2, setae of posterior group of epicranium; SO^1, SO^2, SO^3, setae of subocellar group of epicranium

blunt, spatulate, extremity squarely cut armed with eight stout, rather long, curved spines.

Length of palpus 8.5–9 mm.; breadth at widest part 2.3 mm.

ADULT. ♂♂, ♂; ♀♀, ♂

Antennæ two-thirds the length of the forewings, scheiform flagella, slightly clavate in the male. Labial palpi porrected; second joint long with elongate triangular tuft of projecting scales; terminal shorter, smooth, porrected, pointed; partly hidden by the scales on second joint; reddish ochreous with the underside and inner side white. Maxillary palpi not half as long as labial palpi, porrected, terminal joint ending in small spreading tuft of reddish ochreous scales, tongue long, spiraled, white scales on base. Eyes large and prominent, black. Head rather conspicuous, set in black. Face and head reddish ochreous edged with whitish ochreous. Thorax reddish ochreous. Forewings (fig. 3, C) smooth, elongate, triangular, apex pointed, termen nearly straight; no lines; border veins, 5 and 3 stalked, 10 closely approximate throughout, 7 closely approximate at base, 4 and 3 approximate, 2 slightly before angle of cell, 1b simple; reddish ochreous with faint blackish fuscous markings; a subcostal and angulated, transverse blackish fuscous line crosses the wing from just beyond the middle of costa and edges of an "S"-shaped blackish spot on the end of the cell; another somewhat transverse line begins at apical third of costa; strongly angulated round half outwards, curved on dorsal half, a dark fuscous spot on the middle of the cell, and a series of small black marginal dots along terminal edge. Cilia ochreous with a basal and medial fuscous line. Hind wings (fig. 3, C) broader than forewings, costa straight, termen and dorsum evenly rounded; 8 veins; 4 and 3 closely approximate at base, remote or short stalked; 2 approximate to 1, 7 and 6 stalked, 5 anastomosed with 7 beyond the cell, 4 approximate to 3 in their ends; lower vein of cell without pecten; whitish ochreous, shaded with two fuscous dorsal spots and an oblique, transverse fuscous basal spot. Abdomen whitish ochreous, legs whitish, hind tibiæ with outer median spur absent.

LIFE HISTORY AND HABITS[1]

ADULT STAGE

Emergence

When the moth is about to emerge from the pupal case the ventral surface splits along the margins of the wing sheaths so as to permit the moth to make its exit. This operation requires approximately 4 to 5 minutes. At first the wings appear very small and distorted, but after some fluttering they become very active, almost bellowslike in appearance, and gradually become flattened out until they are fully expanded. During this process the moth has been observed to crawl about at times, although usually it remains rather inactive. Finally the wings relax and come to rest perpendicularly over the back, but they are still soft and limp, and about 45 minutes elapse before they are fit for flight. The shell (pl. 2, A) that is a wing sheath of fore-middle of an adult, is of a pale brown or ivory color, marked centrally with darker lines, and when at rest assumes a characteristic triangular shape, measuring three-eighths of an inch at its widest part. The usual time of emergence is at night, rarely in a few instances were moths observed to emerge during the daytime, and the maximum emergence appears to occur between 10:00 and 12:00 p. m. Observations as shown in Table I indicate that it takes from 30 to 35 minutes to complete the whole operation of emergence.

TABLE I.—Records on time of emergence of adults of *Polychrosis viteana*

Date	Number moths emerged	Sunset	Time moth pupæ were placed in cage	Time emerged	Time in cage	Remarks
1921						
June 8			9:30 a. m.	11:35 a. m.	2:05 p. m.	
July 11	1	8:07 p. m.	4:45 p. m.	9:12 p. m.	4:27 p. m.	
July 12	1	8:07 p. m.	8:45 a. m.	9:15 a. m.	30 m.	
July 13	1	8:06 p. m.	9:15 a. m.	9:50 a. m.	35 m.	

[1] Most of the observations of the life-history studies were made in the greenhouse under natural temperature and these results were eventually compared with observations taken in the insectary.

Longevity

The adults are relatively long lived, their longevity ranging up to a maximum of 23 days. Out of a total of 205 moths confined with plants in insect-rearing cages 124 died within 10 days, 80 lived from 11 to 18 days, and 1 died on the 23d day. It was found that the average life of a female was from 9 to 10 days, while that of a male was only 4 to 5 days. Thirteen moths kept in confinement without a plant lived from 3 to 9 days, with an average life of about 6 days.

Activity

The adults are nocturnal in habit, being more active at night, especially at dusk, when they may be observed flying in a zigzag fashion from plant to plant or swarming near the glass under the eaves. During the daytime they show much less activity and remain concealed, resting inconspicuously on the under side of the foliage or under the benches, or in other places where they are least likely to be disturbed; when alighting on a plant they immediately crawl to the under side of the leaves. Observations on the proportion of sexes in the third generation showed that there were about two females to every male, but in several later generations more males emerged.

Egg Deposition

Soon after mating egg deposition commences and may continue for about two weeks, the maximum number being laid during the first few days and the number decreasing rapidly during the following week. In the case of a few individuals which lived three weeks or more, practically no eggs were laid during the last week. The female moth deposits her eggs singly or in clusters (Pl. 2, B) on the under side of leaves during the night, egg laying rarely commencing until 24 hours after emergence.

The maximum number of eggs laid per individual was not definitely determined because the adults would usually die within two days whenever they were confined singly. In two instances only 42 and 80 eggs, respectively, were deposited within five days after emergence and the moths died three days later. It was necessary, therefore, to confine a number of individuals in a cage at one time. Under these conditions similar difficulty was experienced in securing satisfactory averages, as some of the moths would die after being in confinement for several days. The most reliable records were taken from the adults of the third generation and are given in Table II.

TABLE II.—*Egg-laying records for adults of the third generation of Phlyctaenia rubigalis*

Date of emergence	Number of moths emerged	Date of oviposition	Moths alive	Number of eggs deposited
Apr. 25	37	Apr. 26	37	60
26	[a] 29	27	66	307
		28	48	42
		29	43	250
		30	42	174
		May 1–2	41	133
		3	40	344
		4	40	60
		6	27	0
		7	20	0
		9	1	0
		15	0	0
Total				1,370

[a] Sixteen moths removed for experimental use.

In only one case was deposition observed during the day. Fresh plants placed in cages at 9 a. m. and examined in the evening were normally devoid of eggs, but if plants were left in all night many eggs were usually deposited on them. These records were corroborated by actual observations of 20 moths confined with a calendula plant inside a lantern globe during the night of April 27, 1921. Eggs were deposited on both surfaces of the leaf and on the lantern globe at the following hours: 9.30, 9.50, 10.15, 10.30 p. m., and 12.15 and 1.25 a. m. By 4.25 a. m. the moths had become inactive and no more eggs were deposited that day. When ready to lay eggs the female bends the tip of her abdomen toward the leaf surface, after which the tube of the ovipositor is extended. The eggs are then forced out as small translucent droplets which the moth, with the aid of the third pair of legs, flattens out by pressing them so that they assume a scale-like appearance. During this operation the ovipositor is moved with a quick, nervous action from left to right, whereby the eggs are placed in successive rows forming regular clusters or patches (Pl. 2, B) which may contain as many as 19, arranged in double or triple rows. Observations based on a total of 1,933 eggs from 4 successive generations indicate that the number per cluster is normally less than 10.

TABLE III.—*Range of incubation period of eggs of Phlyctaenia rubigalis during six generations*

Generation	Total number of eggs deposited	Incubation period		
		Minimum	Maximum	Average
		Days	*Days*	*Days*
Second	142	5	9	7.0
Third	313	8	10	9.0
Fourth	3,400	5	10	7.5
Fifth	(a)	5	6	5.5
Eighth	570	4	7	5.5
Ninth	142	4	6	5.0
Average				6.58

[a] Few.

EGG STAGE

Incubation

Consulting Table III, it will be seen that the incubation period varies, being from 4 to 10 days in length and averaging 6.5 days. It was also found that all the eggs do not necessarily hatch at the same time. From a total of 50 eggs deposited on a marguerite plant March 10, 17 hatched on March 19, or 9 days later, while the remaining 33 did not hatch until the following day. Another lot of eggs deposited on the same date on other plants began hatching on March 18, or 8 days after deposition.

Development

The time required for the embryonic development of the young larvae was observed to vary from 4 to 10 days, depending, no doubt, on the temperature and other factors. It was difficult to observe the earlier changes without the aid of a microscope, because of the reticulated structure of the shell, which is iridescent. However, several days after deposition the shell becomes more transparent, and the outer margin more distinct. Shortly before hatching the eggs appear more plump, and segmentation can be recognized.

Newly deposited eggs appear to have a vague granular, cellular structure. The outline of the cells soon becomes more distinct, and definite layers of them are grouped in the periphery of the egg. Invagination takes place at one side, and these cells appear to lose their identity and break down, evidently for the purpose of forming the various organs and internal structures of the body. The first conspicuous change observed was the presence of orange colored spots at one end of the egg, which soon developed into reddish-brown mouth parts. Even before the outlines of the head were apparent, the mandibles could be seen, opening and closing. The eye spots can be seen gradually growing darker, and the legs, prolegs, bristles, and dorsal vessel can be distinguished before segmentation is completed. At about the same time movement by the larva appears to rupture the walls of the yolk cells surrounding the body, and forces the contents toward the anterior end of the body, where it is swallowed. After intermittent periods of activity and rest the larva is then ready to hatch.

Hatching

The larva, when ready to hatch, is in a U-shaped position and occupies almost the entire space within the egg. Contact with the eggshell is loosened by backward and forward movements of the body, and the larva retracts its head before forcing the mouth parts against the shell. The mandibles operate with a pincerlike action in tearing the hole through which it later escapes. As soon as the jaws break through, the head and first two segments are thrust out, and the larva employs its posterior legs and setae to good advantage in crawling free from the collapsed shell. From the time when the mandibles first penetrated the shell until the larva crawled away, hatching was observed to require from 4 to 46 minutes.

Observations on hatching from a cluster of eggs show that the larvae may begin emerging one after another but that sometimes two or three may emerge at the same time. In a group of five larvae which hatched March 25, two larvae becoming active at the same time showed a difference of only 20 seconds in time of hatching. The other three hatched at intervals of 15 and 30 minutes later. On April 14, hatching began in a group of seven eggs at 1.54 p. m. and was completed two hours later. The larvae within two eggs, which were partially overlapping, became active at the same time, but interfered to such an extent that one required two minutes longer than the other to get free from the shell. The observations in Table IV show the intervals of time required between the breaking of the shell and the crawling away of the freed larva.

TABLE IV.—*Time spent in the hatching process by larvae of Phlyctaenia rubigalis*

Date	Time of hatching	Minutes	Date	Time of hatching	Minutes
Mar. 18	8.59 a. m. to 9.03 a. m.	[a] 4	Apr. 14	11.19 a. m. to 11.24 a. m.	5
25	11.02 a. m. to 11.10 a. m.	8	14	12.49 p. m. to 12.53 p. m.	4
25	11.02 a. m. to 11.10 a. m.	8	14	12.54 p. m. to 12.59 p. m.	5
25	11.30 a. m. to 11.37 a. m.	7	14	1.54 p. m. to 1.58 p. m.	4
25	11.45 a. m. to 11.52 a. m.	7	14	2.52 p. m. to 3.01 p. m.	9
25	11.45 a. m. to 11.57 a. m.	12	14	2.59 p. m. to 3.03 p. m.	4
25	1.15 p. m. to 2.00 p. m.	45	14	3.00 p. m. to 3.06 p. m.	6
25	2.57 p. m. to 3.06 p. m.	9	14	3.15 p. m. to 3.20 p. m.	5
26	10.35 a. m. to 10.47 a. m.	12	14	3.36 p. m. to 3.40 p. m.	4
26	12.14 p. m. to 1.00 p. m.	46	14	3.52 p. m. to 3.56 p. m.	4
Apr. 13	9.48 a. m. to 9.50 a. m.	[a] 2	14	4.04 p. m. to 4.10 p. m.	6
13	10.36 a. m. to 11.08 a. m.	32	19	3.18 p. m. to 3.23 p. m.	5
14	10.14 a. m. to 10.19 a. m.	5	19	3.23 p. m. to 3.27 p. m.	4
14	10.46 a. m. to 10.51 a. m.	5	19	3.20 p. m. to 3.25 p. m.	5
14	10.56 a. m. to 11.04 a. m.	8	19	3.25 p. m. to 3.29 p. m.	4
14	11.17 a. m. to 11.21 a. m.	4	19	3.27 p. m. to 3.31 p. m.	4

[a] Head and two segments free before time was noted.

LARVAL STAGES

Development

Five larval stages were observed between the time of hatching and pupation. In each case the larvae molted after approximately equal feeding periods. They became inactive usually within a slight shelter of webbing or tied leaves, soon losing their greenish color, and appearing whitish or cream colored before casting their skins. Molting frequently occurred over night after which feeding was soon resumed. The most conspicuous part of the cast skin is the chitinized head covering, which is usually yellowish brown, becoming nearly colorless in the later stages, but in the case of the first molt it is black.

The newly hatched larva (Pl. 2, C) is about a millimeter long and very slender. Its shining translucent, creamy-white color changes to pale green as soon as feeding begins, owing to the food contents which are visible through the body wall. The black color of the head, which is conspicuous during the first larval stage, sometimes persists until the second molt, after which it becomes pale brownish with darker blotches on each side. The body soon acquires the characteristic dark green longitudinal stripes with lighter ones on either side. With successive molts these markings become more pronounced and the tubercles also become more prominent. There is very little change except in size during the later stages. The most striking increase in size appeared to occur during the first and in the final stages.

TABLE V.—*Observations on molting and duration of larval periods of Phlyctaenia rubigalis*

Date of hatching	Dates of molting					Duration of larval stages					Total larval period
	First	Second	Third	Fourth	Fifth	First	Second	Third	Fourth	Fifth	
1921						*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Jan. 28	Feb. 2	Feb. 5	Feb. 9	Feb. 14–15	Feb. 20	5	3	4	5–6	5–6	22–24
Jan. 29	Feb. 3	Feb. 7–9	Feb. 11	Feb. 16	do	5	4–6	2–4	5	4	22
Feb. 3	Feb. 5	Feb. 9	Feb. 14	Feb. 19	Feb. 21–25	2	4	5	5	2–6	18–22
Mar. 17	Mar. 20	Mar. 22–23	Mar. 25	Mar. 29	Apr. 4	3	2–3	2–3	4	6	18
Mar. 18			Mar. 28	Apr. 1	Apr. 6	3			4	5	19
Mar. 26	Mar. 29	Apr. 4				3	6				
Apr. 14	Apr. 17					3					
Apr. 19	Apr. 23					4					
Apr. 20	do					3					
Apr. 30	May 3	May 6	May 10–11			3	3	4–5			
May 3	May 7	May 12				4	5				
May 5	May 9					4					
Nov. 1	Nov. 5	Nov. 8–10				4	3–5				
Nov. 3	Nov. 7	Nov. 10		Nov. 19	Nov. 23	4	3			4	20
Nov. 15	Nov. 18	Nov. 21			Nov. 30	3	3				15

As illustrated in Table V, the first molt may occur from 2 to 5 days after hatching, and is usually followed by a second molt from 2 to 6 days later, at which time the black heads disappear and the larvae are approximately 2.5 mm. long. After feeding from 2 to 5 days the larva molts for the third

time. In the fourth stage, which lasts from 2 to 6 days, they are usually 7 to 9 mm. in length. The fifth stage also requires from 2 to 6 days and is concluded by the fifth molt just prior to pupation; the larvae in this stage are from 14 to 18 mm. long, but when full-grown they are about 18 mm. long (Pl. 2, D, E). Observations based on 171 larvae representing five generations indicate that it requires from 15 to 30 days, with an average of about 21 days (see Table VI), to complete the larval development.

TABLE VI.—*Duration of larval period of Phlyctaenia rubigalis*

Generation	Number of larvae	Duration of larval period	
		Minimum	Maximum
		Days	*Days*
Second	25	22	30
Third	82	17	28
Fourth	2	17	21
Eighth	36	18	23
Ninth	26	15	20

Larval coloration varies with the kind of plant on which the larva is feeding. On cinerarias the larvae are more yellowish-green than on marguerites, and on primulas they acquire a bright-green color; but the relation is most pronounced in the case of coleus which gives them a dull reddish-brown appearance.

Feeding Habits

The larvae upon hatching show more or less activity and move about on the surface of the foliage, apparently in search of an abrasion or place to begin feeding; they remain, however, grouped on the leaf upon which eggs were deposited and from which they hatched. They usually feed on the lower side of the leaf, or in the rolled leaf, or in leaves webbed together for their protection. When feeding their heads are bent at almost right angles to the body and small pieces of the leaf are gnawed out from circular holes which are enlarged as the larvae continue to feed along the edges. In the later feeding the small areas coalesce, resulting in the destruction of the entire leaf surface upon which they are feeding. There is a marked change in the size of the larvae between the time of hatching and a few hours after feeding. In some instances they become at least four times their original size. As newly hatched larvae they apparently have some difficulty in beginning a feeding area, and it has been noticed that they tug, tear, pull, or dig at the leaf surface in order to break through the tissues, but whenever an abrasion occurs it is quite promptly made their point of attack.

The exact type of injury varies somewhat in the case of different host plants. On cinerarias and marguerites they skeletonize the leaves, usually feeding on the lower surface, and on the latter they also draw the leaves together, while on the rose they appear to chew small pieces of the under surface of the leaves. They ate small round holes through violet leaves or else they fed on the under surface, leaving only the epidermis of the upper surface intact, giving it a blotched appearance. Even though the rose is reported as one of its favorite hosts in some sections, several attempts to rear larvae on it in the laboratory failed since they would invariably spin silken strands to lower themselves from the plant, and crawl away.

During periods of rest the transparency of the body tissues permitted observations on the complete process of digestion through the alimentary canal. The young larvae when disturbed suspend themselves from the leaf by spinning a silken strand on which they sway backward and forward, clinging to any object which they may encounter. They are able to cross gaps from one leaf surface to another by stretching or reaching across, resting on the prolegs during the operation. Feeding has been observed on the sides of the veins and tips of the leaves, and at times they may eat a narrow strip along the under side of the leaf and afterward draw it together with webbing.

Web Making

As a general rule web making or spinning does not take place until after the larvae begin feeding. In the case of a larva hatched four hours before, the following procedure was observed: The anterior portion of the body swayed backward and forward, evidently in search of a resting place for the legs while hanging on with the prolegs, the head and first anterior segment moving together, with only slight motion of the second and third segments. Since strands of silk were noticed emanating from the mouth, these actions were apparently associated with web making. The first pair of legs was used in attaching webbing to the tips of the leaf hairs. A single strand was first made, then several others were joined to it at a central point to form a web surface, similar to a spider web. As soon as a small area of the leaf surface had been covered with webbing to afford protection and support, the larva devoured a portion of the leaf tissue equal to the size of its head. It

then resumed spinning, later continuing to feed in the same hole and consuming about the same amount each time. After sufficient webbing had been formed, the larva crawled under it and fed at intervals, with intermittent periods of inactivity.

In another instance a larva, while hatching, constructed webbing with diagonal and cross strands, which it appeared to use as a foothold or brace in extricating itself from the eggshell, and as a path on which to crawl out.

Webbing is associated with feeding during the several stages, and especially at the time of molting. Just prior to pupation the full-grown larva spins a slight silken cocoon within which the pupa transforms to the moth.

PUPAL STAGE

Pupation takes place in folded leaves, except when the plant is so badly skeletonized that the larva crawls off to seek the shelter of some crevice or protected place. When the full-grown larva is ready to pupate, it forms a hiding place in one of the following ways, depending on the kind of leaves available: On geraniums it sometimes cuts each side of a portion of the leaf and folds it back, or it curls the edge of a leaf under, or laps a portion of the leaf surface which it fastened on the lower surface; and on marguerites and chrysanthemums it rolls over portions of the leaf so carefully that it is difficult to detect the presence of the pupa within.

The larva, which lines the leaf with a loose web of rather tough, white, silken strands, remains inside until it has transformed to the moth and is ready to emerge. It soon loses its characteristic greenish appearance, becoming distinctly cream colored or yellowish, and its body contracts, so that it is much shorter and thicker. This prepupal stage was observed to last less than a day. The pupal case, within which transformation takes place, is soon formed, being light colored at first but usually growing darker in a few hours. With the exception of occasional lighter and darker specimens the majority of the pupae have a shining chocolate-brown color (Pl. 2, F).

Observations on 142 individuals, as shown in Table VII, representing six generations, indicate that it requires from 6 to 16 days, with an average of about 10 days, for the pupal period.

TABLE VII.—*Duration of pupal stage of Phlyctaenia rubigalis*

Generation	Number of pupae	Duration of pupal stage	
		Minimum	Maximum
		Days	*Days*
First	7	10	12
Second	30	11	16
Third	54	7	15
Fourth	2	9	
Eighth	23	6	10
Ninth	26	8	10

SEASONAL HISTORY

TABLE VIII.—*Length and sequence of life cycles of Phlyctaenia rubigalis, 1921*

Generation	Number of specimens	Dates of—		Length of life cycle
		Egg deposition	Adult emergence	
				Days
First	7		Jan. 17 to 20	(a)
Second	5	Jan. 23	Mar. 4 to 6	40–43
	8	Jan. 23	Mar. 7 to 8	43–44
	2	Jan. 25	Mar. 8 to 9	42–43
	7	Jan. 25	Mar. 9 to 14	43–48
	1	Jan. 23	Mar. 24	60
Third	8	Mar. 8	Apr. 15 to 16	38–39
	12	Mar. 9	Apr. 17 to 20	39–42
	31	Mar. 9	Apr. 21 to 24	43–46
	10	Mar. 9	Apr. 25 to 29	47–51
	12	Mar. 10	Apr. 15 to 16	36–37
Fourth	(b)	Apr. 18 to May 2	May 26 to June 16	38
Fifth	(b)	May 27	July 1 to 15	35
Sixth	(b)	July 2	Aug. 10	39
Seventh	11	Aug. 10	Sept. 14 to 17	35–38
	21	Aug. 12	Sept. 18	37
	41	Aug. 13	Sept. 19 to 23	37–41
	5	Aug. 17	Sept. 24	38
Eighth	2	Sept. 19	Oct. 24 to 26	35–37
	6	Sept. 22	Oct. 31 to Nov. 4	39–43
	5	Sept. 23 to 24	Nov. 7 to 8	45
	2	Sept. 26	Nov. 8 to 9	43–44
	5	Sept. 27 to 29	Nov. 9 to 10	42–43
	3	Sept. 30	Nov. 8	39
Ninth	4	Oct. 29	Dec. 2	34
	5	Nov. 1	Dec. 2	31
	12	Nov. 9	Dec. 8 to 9	29–30
	5	Nov. 10	Dec. 10 to 12	30–22

[a] Partial life cycle.

[b] Specimens not confined.

As may be observed in Table VIII nine distinct generations were reared during 1921, indicating that there may be a definite overlapping of generations throughout the year under greenhouse conditions. The first seven moths, which began emerging January 17 from nearly full-grown larvae received January 5, were considered as completing a partial first generation. Twenty-three adults of the second generation began appearing March 4. The life cycle of 73 moths was observed during the third generation and the first individuals emerged on April 15. Observations on the fourth, fifth, and sixth generations were made on specimens reared under normal greenhouse conditions because the high temperatures prevailing during June, July, and August made it difficult to rear them in cages. The first adults of the seventh, eighth, and ninth broods appeared on the following dates: September 14, October 24, and December 2, with totals of 78, 23, and 26 specimens, respectively. The time between emergence of the first adults of the ninth generation and January 5 of the ensuing year would allow sufficient time for the completion of the partial first generation mentioned above. Like other insect pests which live both indoors and outdoors, the greenhouse leaf-tyer apparently flourishes best under glass during the winter and spring months, at which time its depredations are most frequently reported. In the summer months it causes serious injury outdoors on truck and garden crops, especially on celery, beets, and lettuce.

NATURAL ENEMIES

In the course of these life-history studies, spiders and ants proved to be important enemies of *Phlyctaenia rubigalis*. Small spiders of the species *Theridium tepidariorum* Koch, which conceal themselves along the sides of the flowerpots, or even on the leaves of infested plants, confined their attention to destroying the larvae and adults, while the pupae were apparently unmolested. The spider first renders its victim helpless by entangling it with a few strands of webbing and then puncturing it and later sucking the entire body contents.

The small red ant sometimes called Pharaoh's ant, *Monomorium pharaonis* L., was not observed to disturb the eggs but attacked all other stages, being especially destructive to the pupae and moths. These ants chewed the pupae into bits and devoured them, and not only ate the bodies of the moths, but even carried away pieces of the wings.

No insect parasites were reared from any of the material used in these experiments, but the following natural enemies have been recorded as occurring in the United States:

Chittenden (*3, p. 19*) says: "A single parasite of this species has been observed, the only natural enemy that appears to be known for it. Among a lot of larvae from Livonia, Pa., a cocoon was found May 19, which gave the imago May 27. It was identified by Mr. Ashmead as a species of Synetaeris, an ichneumonid genus related to Limneria."

J. J. Davis (*4, p. 100*) reports: "A hymenopterous parasite of this leaf-tier was common in the greenhouses of Chicago, and no doubt in many of them it was doing much to hold the leaf-tier in check. The species was determined by Chas. T. Brues as *Apanteles glomeratus* Linn. A tachinid fly bred from this leaf-tier was determined by C. A. Hart as *Phorocera parva* Bigot."

According to Gibson (*6, p. 629*) no parasites were reared from Canadian material.

CONTROL

Since a review of published records and recent correspondence relating to this pest indicates that none of the recommendations for control have proved entirely satisfactory, experimental work was conducted that further data on this subject might be obtained. These tests included fumigation with hydrocyanic-acid gas and dipping, spraying, or dusting with insecticides.

FUMIGATION WITH HYDROCYANIC-ACID GAS

Although some writers have suggested the use of hydrocyanic-acid gas, its use has generally been discouraged because burning of the tender growth frequently has accompanied such treatment when employed at sufficient concentration to kill the moths. Recent experience, however (*12*), indicates that although slight temporary injury may occur on such plants as jasmine, ageratum, German ivy, chrysanthemum, marguerite, salvia, geranium, dahlia, cestrum, heliotrope, and stephanandra, no permanent injury will result. In the case of violets, Chittenden (*3, p. 20*) recommends it as a most satisfactory remedy, but he also says that "it can not yet be safely used for the fumiga-

tion of certain other plants owing to the danger of bleaching and otherwise injuring them." In a footnote he states that Slingerland (*13, p. 163–164*) "tested this gas against this leaf-tyer, and his experiments show that the gas does not kill moths, pupae, and grown larvae when used at the ordinary strength, but only small larvae."

Britton (*2, p. 370*) recommends fumigation with this gas at the rate of 0.1 to 0.15 grams of 98 per cent potassium cyanide for each cubic foot of space (or 3.5 ounces per 1,000 cubic feet). Expressing this dosage in terms of sodium cyanide (which is now generally used for fumigation purposes), it would be equivalent to 2.64 ounces of sodium cyanide, an amount greatly in excess of dosages which can be safely employed for general greenhouse fumigation.

Moreover, results of recent experiments indicate that the egg, larval, and pupal stages are not affected by fumigation with dosages ranging from one-fourth to one-half ounce sodium cyanide per 1,000 cubic feet of space that are ordinarily employed.

To throw more light on this point the experiments recorded in Table IX were undertaken with plants harboring all stages of the leaf-tyer.

From the results obtained it is quite clear that the moths ultimately succumb to the above dosage of the gas, and may be effectively controlled provided the plants concerned can tolerate it. Furthermore, no egg deposition took place from the time of exposure to the gas until the death of the moths. The larvae and pupae developed normally and were not affected by this treatment. Moreover, pupae placed in a cyanide bottle from one-half hour to 24 hours emerged later as normal moths. All controls developed normally.

Incidentally, fumigation with tobacco has proved ineffective against any and all stages of the insect.

TABLE IX.—*Fumigation with hydrocyanic-acid gas for the control of all stages of Phlyctaenia rubigalis* [a]

Experiment No.	Date	Number of specimens	Observations and results
	1921		
I	Apr. 6	36 moths	Apr. 7, 12 moths dead, 24 alive. Apr. 8, 21 moths dead, 3 alive. Apr. 9, all moths dead.
		10 larvae	No effect, all developed normally.
		5 pupae	No effect, all emerged normally.
		15 eggs [b]	No effect, all hatched normally.
Control	Apr. 6	4 moths	Normal, egg depostion continued.
		60 larvae	Normal, developed to pupae.
		15 pupae	Normal, developed to adults.
		59 eggs	Hatched normally.
II	Apr. 25	15 moths	Apr. 25, at close of fumigation all moths were lying at bottom of cage. Apr. 26, 9 a. m., 14 moths alive, 1 dead. Apr. 26, 3.45 p. m., 5 moths alive, 9 dead. Apr. 28, 9 a. m., all dead.
		100 eggs	No effect, all hatched.
Control	Apr. 25	144 moths	Normal development.
		800 eggs	Normal hatching.

[a] In each of these experiments 1 ounce of sodium cyanide per 1,000 cubic feet of space was used with an exposure to the gas lasting 1 hour, and at temperatures of 73° F. and 76°, respectively.

[b] These eggs were deposited by the moths just prior to fumigation.

TABLE X.—*Results of tests to determine residual insecticidal action as well as the immediate effect of contact insecticides on eggs of Phlyctaenia rubigalis*

Experiment No	Date sprayed	Dosage and treatment	When treated	Number of eggs	Date of—		Observations and results
					Deposition	Hatching	
	1921						
I	Apr. 25	40 per cent nicotine sulphate, 1–800; no soap; entire plant dipped.	Before deposition.	190	Apr. 26	May 3	May 3, no effect on hatching; lower leaves died, possibly owing to dipping; larvae fed heavily, and 9 days later plant was ruined.
II	do	Same as in Experiment I.	After deposition.	55	Apr. 24	Apr. 30	Apr. 30, eggs hatched.
					Apr. 29		May 12, all larvae developing normally.
III	do	Soap solution, 1 ounce to 1 gallon water; 2 plants dipped.	Before deposition.	19	Apr. 26	May 4	May 4, no effect on hatching; lower leaves died; larvae fed heavily.
				24	do	May 5	May 12, plant ruined as result of feeding.
IV	do	Same as in Experiment III.	After deposition.	110	Apr. 25	May 3	May 3, eggs hatched; no effect on eggs or larvae.
							May 9, larvae fed so heavily that leaves were badly spotted.
V	do	40 per cent nicotine sulphate, 1–800; soap, 1 ounce to 1 gallon; plants dipped. For egg deposition at intervals of 1, 3, and 5 days, respectively.	Before deposition.	38	Apr. 26	May 4	May 4–10, no effect on hatching; larvae fed and developed normally. Eggs deposited Apr. 28–30 showed tendency to scale loose, but later hatched.
				45	Apr. 28	May 7	
				40	Apr. 30	May 10	
VI	do	Same as in Experiment V.	After deposition.	44	Apr. 25	May 3	May 3–10, eggs hatched; lower leaves showed slight injury; larvae developing normally.
					May 1–2	May 10	
VII	do	Control; no treatment.	Before deposition.	139	Apr. 29	May 8	May 8, all hatched and developed normally.
VIII	do	Control; no treatment.	After deposition.	90	Apr. 24	Apr. 30	May 8, all hatched and developed normally.

TESTS FOR THE DESTRUCTION OF EGGS BY DIPPING PLANTS IN CONTACT INSECTICIDES BEFORE AND AFTER EGG DEPOSITION

In some previous experimental work (*11, p. 17–18*) with insecticides, it was observed that a residual or continued insecticidal action often operates against eggs subsequently deposited on foliage which has been treated. Accordingly plants were dipped both before and after deposition of eggs, contact poisons being used as indicated in Table X. In this way data were obtained on the residual insecticidal action as well as the direct effect of contact insecticides on the eggs.

These tests proved negative so far as throwing any light on the residual or immediate contact effect of insecticides on eggs of the greenhouse leaf-tyer.

TESTS FOR THE DESTRUCTION OF EGGS BY SPRAYING PLANTS WITH ARSENICALS BEFORE AND AFTER EGG DEPOSITION

Further tests were then made, arsenicals being substituted for the contact sprays, as indicated in Table XI, it being assumed that the young larvae would naturally feed on the poisoned foliage after hatching.

Arsenicals, either alone or combined with nicotine sulphate, proved only partially effective against the eggs, but as soon as the larvae hatched and fed on the arsenical-coated foliage, they died. Although burning was evident in one case, subsequent experiments showed that the plants tolerate this treatment. The whitish deposit which remains on foliage so treated may be objectionable from a commercial point of view, but when applied to young plants this deposit is left behind as growth continues.

TABLE XI.—*Results of tests to determine effect, on eggs of Phlyctaenia rubigalis, of spraying plants with arsenicals before and after egg deposition*

Experiment No.	Date sprayed	Dosage and treatment	When sprayed	Number of eggs	Date of—		Observations and results
					Deposition	Hatching	
	1921						
I	Apr. 25	Lead arsenate, 1 ounce to 1 gallon water.	Before deposition.	46	Apr. 26	May 3	May 3, no effect on egg stage; all larvae dead or missing 4 days later; severe burning on tips of plants.
II	do	Same as Experiment I.	After deposition.	46	Apr. 24	Apr. 30	May 2, 8 eggs failed to hatch; larvae feeding, but disappeared May 3.
				49	Apr. 29	May 9	May 3, all disappeared.
III	do	do	Before deposition.	49	do	May 7	May 7, some eggs failed to hatch; no live larvae on plant 5 days later.
IV	do	Same as Experiment I, plant sprayed just before eggs hatched.	After deposition.	89	Apr. 17	Apr. 25	May 26, a few larvae dead; some alive, but suspended from leaves. May 27, 8 larvae dead. May 29, all larvae dead.
V	do	Lead arsenate, 1 ounce to 1 gallon water; 40 per cent nicotine sulphate, 1–800.	Before deposition.	73	Apr. 26	May 4	May 3, some eggs scaled loose from leaf; a few dried and failed to hatch. May 9, no larvae remained; no injury to plant.
VI	do	Same as Experiment V.	After deposition.	28	Apr. 24	May 2	May 2, eggs hatched.
				78	May 1–2	May 9–10	May 9, all larvae dead.
VII		Control; no treatment.		90	Apr. 27	May 4	May 4, eggs hatched and larvae developed normally.
VIII		do		44	Apr. 25	May 3	May 3, all larvae developed normally.

TABLE XII.—*Control of larvae of Phlyctaenia rubigalis by spraying with or dipping in arsenate of lead*

Experiment No.	Treatment, Apr. 8, 1921	Total number of larvae	Number of dead larvae	Date of death	Observations and results
				1921	
I	Plant sprayed with lead arsenate, 1 ounce, powdered, to 1 gallon water.	28	21	Apr. 9	Apr. 16, 24 dead, 4 disappeared.
			2	Apr. 11	
			1	Apr. 12	
II	Plant dipped in lead arsenate, 1 ounce, powdered, to 1 gallon water.	33	22	Apr. 9	Apr. 20, 27 dead, 6 disappeared.
			3	Apr. 11	
			1	Apr. 18	
			1	Apr. 20	
III	Plant dipped in lead arsenate, 1 ounce, powdered, to 1 gallon water, plus 40 per cent nicotine sulphate, 1–800.	20	12	Apr. 9	Apr. 20, 16 dead, 4 disappeared.
			3	Apr. 11	
			1	Apr. 20	
IV	Control; no treatment	12	0		Larvae developed normally; pupated April 16.

SPRAYING AND DIPPING TESTS FOR THE CONTROL OF LARVAE WITH ARSENATE OF LEAD

Further tests were then conducted to determine the effectiveness of spraying plants with arsenate of lead, or dipping them in this arsenical, for the control of partially grown larvae. The results of these experiments are given in Table XII.

DUSTING TESTS FOR THE CONTROL OF LARVAE

The results obtained by dusting with the arsenical-sulphur mixtures, as shown in Table XIII, were gratifying and hardly need further comment, except the statement that in the earlier tests a few individuals crawled away from the treated plants and were lost, because the plants were not confined within cages. Without exception, however, when such specimens were located they were dead, and were easily identified by the characteristic arsenical poisoning displayed by them when succumbing to this insecticide. The larvae, after feeding on the dusted foliage, soon lose their normal greenish color, becoming sluggish and limp. After death their bodies are rather soft and have a sickly yellowish color.

Results of these tests show a definite control, indicating that timely spraying with or dipping in arsenate of lead is effective in killing the larvae.

DUSTING TESTS FOR THE CONTROL OF THE MOTHS

During the spring of 1924, in preliminary experiments, nicotine dusts containing 5 per cent of nicotine sulphate (2 per cent pure nicotine) proved effective in killing the moths. In these tests the specimens were confined in wire cages and the dust was applied with a small hand duster. The dusts were prepared by impregnating hydrated lime with a 40 per cent nicotine sulphate solution according to formula No. 2, on page 5 of Farmers' Bulletin 1282.

TABLE XIII.—*Control of larvae of Phlyctaenia rubigalis by dusting*

Experiment	Date	Plant	Treatment	Total number of larvae	Number of dead larvae	Date of death	Observations and results
	1921						
I	Apr. 8	Cineraria	Lead arsenate, 10 per cent; sulphur, 90 per cent.	58	26	Apr. 9	42 dead larvae; 6 alive; 10 disappeared; 72 per cent kill.
					13	Apr. 11	
					1	Apr. 12	
					2	Apr. 16	
II	Apr. 8	Marguerite	Same as Experiment I.	13	1	Apr. 9	1 larva dead; 11 disappeared; 1 pupated.
III	May 13	Calendula	do	23	17	May 14	17 dead; 5 disappeared; 1 alive; 74 per cent kill.
						May 17	
IV	May 16	do	do	19	3	May 17	May 23: 100 per cent kill.
					8	May 18	
					6	May 19	
					2	May 23	
V	May 16	do	Calcium arsenate, 10 per cent; sulphur, 90 per cent.	41	6	May 17	40 larvae dead within 1 week; 1 pupated (plants treated 3 days before larvae were due to pupate); 97 per cent kill.
					16	May 18	
					5	May 19	
					5	May 20	
					7	May 23	
					1	May 24	
VI	May 12	Marguerite	Lead arsenate, 15 per cent; sulphur, 85 per cent.	36	32	May 13	100 per cent kill; larvae 1 week old; all dead within 40 hours after dusting.
					4	May 14	
VII	May 12	do	Same as Experiment VI.	23	20	May 13	100 per cent kill.
					3	May 14	
VIII	May 13	Calendula	Lead arsenate, 20 per cent; sulphur, 80 per cent.	25	20	May 14	May 14, 20 larvae 1 week old killed; 4 alive but later disappeared; 80 per cent kill.
IX	May 16	do	Same as Experiment VIII.	13	6	May 17	100 per cent kill.
					1	May 18	
					5	May 19	
					1	May 20	
X	May 12	do	Lead arsenate, 10 per cent; tobacco dust, 10 per cent; sulphur, 80 per cent.	35	26	May 13	35 larvae 2 weeks old killed; 100 per cent kill.
					3	May 14	
					3	May 16	
					3	May 17	

Even though there has been no opportunity to test this treatment on a commercial scale, the indications are so promising that it seems desirable to include the use of nicotine dust in the control program. The plants are not injured, and the moths and some of the younger larvae hit by the dust are killed. Moreover, because of the ease of application many florists will no doubt prefer to use the dust rather than fumigate with hydrocyanic-acid gas.

SUMMARY AND CONCLUSIONS

The preceding data bring out certain features which may be used as a basis upon which to formulate a definite control program. Briefly summarized, the life-history studies show that this insect breeds practically without interruption, and that all stages are likely to be found in greenhouses throughout most of the year. The destruction of plants is due to the feeding of the larvae, which later increase the injury by tying themselves up in the foliage prior to pupation, thus causing further disfigurement and consequent decrease in commercial value. The pupae are well protected by the formation of the pupal case within tied leaves lined with a rather tough silken cocoon. The adults are nocturnal in habit.

The results of the control experiments indicate (1) that the adults may be successfully controlled by fumigation with hydrocyanic-acid gas, or by dusting with a 5 per cent nicotine sulphate dust; (2) that the eggs are impervious to contact insecticides; (3) that the larvae succumb to the effects of arsenical poisons when applied either in the dry or liquid form, and (4) that the pupae are virtually immune from all artificial control measures, except hand picking, because of their protection and resistance. It is evident, therefore, that an effective control program necessitates simultaneous direction of efforts against the susceptible stages, which are the adults and larvae.

RECOMMENDATIONS

In case of a heavy existing infestation the following drastic control measures are recommended: Fumigate after dusk twice at intervals of seven days with hydrocyanic-acid gas, using 1 ounce of sodium cyanide per 1,000 cubic feet of air space, with an exposure lasting for one hour. The first fumigation will operate against all moths which are present at the time of fumigation, and the second will kill all moths which have emerged since. The day following the first fumigation the plants should be treated with the arsenical insecticides as outlined in the paragraphs below.

When it is not feasible to fumigate with hydrocyanic-acid gas, persistent dusting with a 5 per cent nicotine sulphate dust is recommended.

For plants which are well developed and of large size, or growing in permanent beds, dusting with a dry mixture consisting of 1 part of calcium arsenate or arsenate of lead and 9 parts of superfine sulphur is very effective, and should be applied by means of a modern type of hand duster or blower gun. This treatment has the advantage of sifting the insecticide through the webbing which the larvae spin, as well as poisoning the leaf surface on which they feed. It possesses the additional advantage of overcoming the unsightly deposit which follows the application of liquid arsenical sprays.

For low-growing or potted plants, spraying with lead arsenate or calcium arsenate solution, prepared by dissolving 1 ounce of the powdered form in each gallon of water used, is recommended. Spray solutions are desirable for the treatment of the smaller plants because the entire plant may be dipped directly in the solution or else sprayed with a knapsack or compressed-air sprayer, an angled nozzle being used so that the spray can be directed to all surfaces of the infested foliage.

The most important considerations in controlling the larvae or caterpillars are promptness and thoroughness in the applications of the poison, especially to the under side of the leaves where the larvae feed. Since the young larvae are less resistant to the insecticides soon after hatching and before they become webbed-in on the leaves, they can be more easily destroyed at this stage and, moreover, there is less risk of the plants becoming seriously disfigured. After the webbing is formed it is difficult to coat the foliage with a liquid spray because the silken fibers prevent the insecticide from reaching the leaf. It is also imperative that the plants be kept constantly covered with the spray.

In case of a light infestation, or one which is discovered in its incipiency, going over the plants daily and picking and destroying any that may be found will prove effective.

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A LEAF AND CORM DISEASE OF GLADIOLI CAUSED BY BACTERIUM MARGINATUM [1]

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INTRODUCTION

The disease caused by *Bacterium marginatum* probably occurs to some extent wherever gladioli are grown, but in some regions it does not attract much attention because the signs of disease are inconspicuous or entirely lacking. In other localities with more favorable conditions for the parasite, the loss may be considerable. The parasite in viable and pathogenic condition is carried over on the corms from season to season. The lesions being sometimes small, may escape detection; therefore corms apparently normal may be the means of carrying the disease to various parts of the country where, if conditions favor its development, it may become a menace to gladiolus culture.

The bacteria causing this disease have been isolated from gladioli grown in Florida, California, Virginia, Pennsylvania, Indiana, Michigan, Maryland, and the District of Columbia. This disease was first studied by the writer in 1913 when a commercial grower in the Middle West sent to the United States Department of Agriculture a number of infected plants, and stated that the disease was causing considerable loss in his fields. From these plants an organism was isolated which proved to be definitely pathogenic and produced lesions on inoculated gladioli identical with those on the original host. The cultural characters of the organism were also determined at this time.

In 1920 the disease was observed on gladioli growing in the District of Columbia and Virginia. Plants grown on the same land and from corms produced in 1920 were under observation from 1921 to 1923 and all showed a recurrence of the disease.

Several gladiolus farms in Central Michigan were examined by the writer in September, 1922, and the disease found generally prevalent but, according to the growers, it was less severe than in some previous years.[2]

A reference to laboratory notes indicated that an organism with similar characters was isolated from diseased gladioli received in April, 1910, from California. In July, 1922, the writer found inconspicuous lesions of this disease on gladioli in a florist's shop in Los Angeles, Calif. The disease was not found at that time in the plantings about Los Angeles.

A brief description of this disease and of the causal organism (*Bacterium marginatum*) was published in 1921 (*1*).

In Phytopathology (*2*) there is an abstract of a paper read at the Cincinnati meeting. This abstract deals not only with the disease caused by *Bacterium marginatum* but also with another bacterial leaf blight of gladioli (*3*) caused by *Bacterium gummisudans*.

DESCRIPTION OF THE DISEASE

The lesions may appear on any part of the leaf but are usually confined to the fleshy, basal region, and it is here that the serious damage occurs. Tiny specks of bright reddish-brown color, slightly elevated and usually in considerable numbers, are the first visible signs of the disease. By enlarging and coalescing these spots produce large necrotic areas, in which all the parenchyma tissue is destroyed (Pl. 1). When infections are more isolated, very typical lesions are produced. The tiny speck enlarges to a circular, or more often, elongated spot; the center is sunken, dark-brown, or almost black with the margin slightly elevated and

[1] Received for publication June 25, 1924—issued January, 1925.

[2] Thanks are given to H. C. Oven of Ovid, Mich., for cooperation in collecting specimens of diseased plants, supplying healthy corms for experimental purposes and for many helpful suggestions.

darker in color. (Sanford's brown to hazel brown for lightest color and mahogany brown to black for the darkest (*7*).[3] These spots look quite like burned places, the margins especially resembling charcoal. The fibrovascular bundles are not primarily attacked and stand out prominently in the sunken areas. Ordinarily the infected tissues are rather dry and firm, but if the plants are kept under unusually moist conditions the disease progresses rapidly like a general soft rot and without the brightly colored, definitely outlined spots.

Infection is most general on the lower parts of the leaves, from ground level up to a height of 6 to 8 inches. Under favorable conditions the spots enlarge rather rapidly and soon destroy large areas of the soft tissues. The whole thickness of the leaf is involved, and protected from drying influences by the outer leaves, the rot progresses more rapidly in the interior and has here more of the appearance of a soft rot. Plants showing but slight exterior signs of disease often show a surprising amount of rot when the leaves are pulled apart. The underground stem-like part just above the corm usually shows evidence of the disease, either as tiny reddish-brown spots or as a more extensive rot.

So long as the fibrovascular system functions the leaf parts above the lesions remain normal. The vascular system seems not to be directly affected, but eventually the vessels become blocked with a brownish, gum-like substance and the leaf parts above die. Sometimes only a narrow, dry, brown streak is present in a leaf; more often the whole leaf is brown and dry. In some cases one or more brown leaves are seen in plants otherwise normal in appearance (Pl. 2).

In cases of severe infection, with destruction of large areas of cellular tissue, the whole plant falls over, bending at or near the ground level. At this stage of the disease the basal parts are dull brown in color, fibrous, and soft-rotted or dry-rotted, depending on the amount of moisture present (Pl. 1, A).

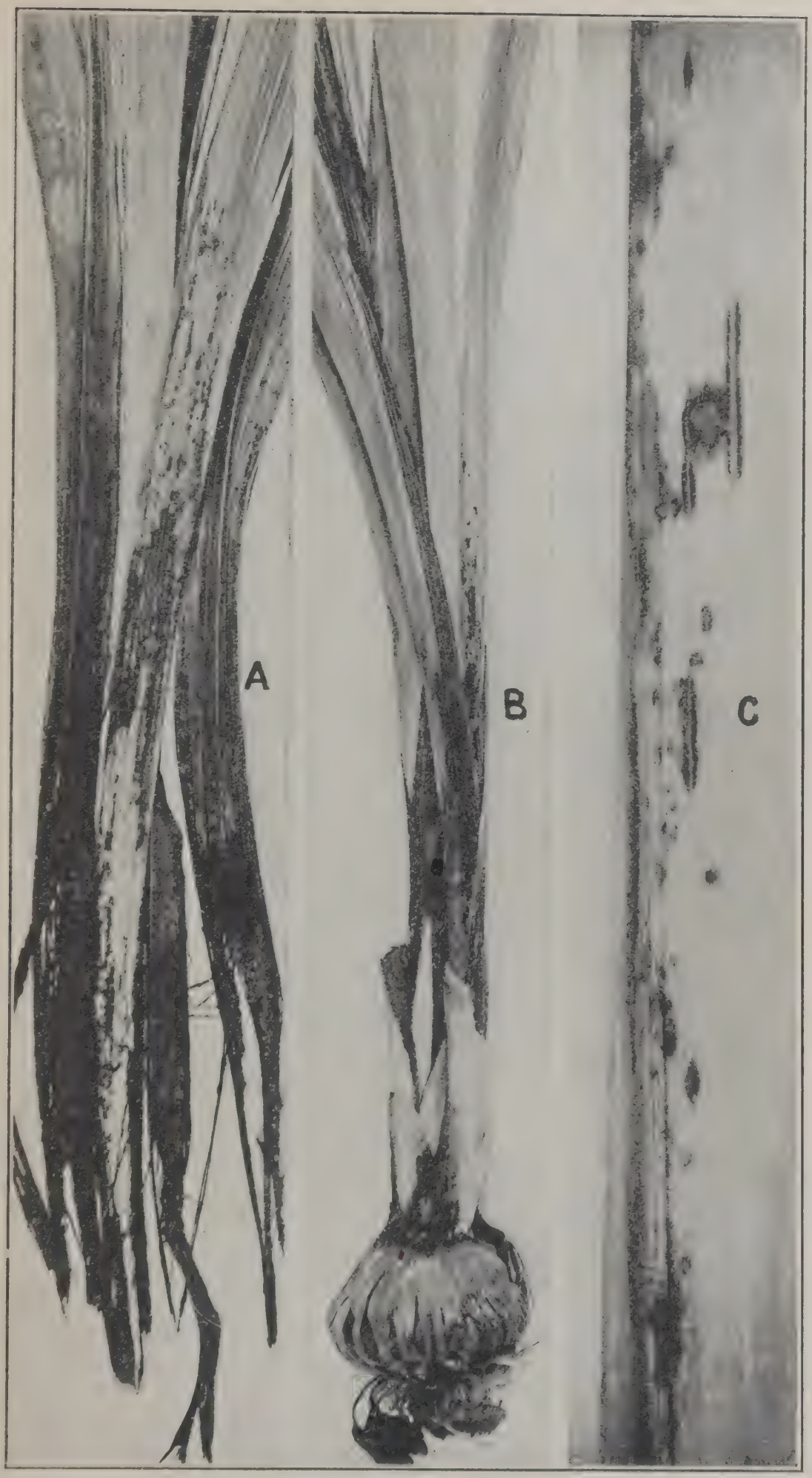
The upper parts of the leaves have but few infections. These remain small and cause no damage except to the appearance of the foliage (Pl. 4, F).

The date of the appearance of the disease depends largely on the temperature and moisture conditions. Warm, moist weather seems a necessary factor for infection. In the vicinity of Washington, D. C., the earliest noted appearance was June 6, 1921, on the variety America. The plants, about 1,300 in number, were from corms that had remained in the ground all winter. In 1920 the plants in this bed were moderately infected with the disease. The 1921 shoots came up early in April and remained entirely normal, clean, and vigorous until June 6, when the first small but typical spots were found. Isolation plates from these spots produced the characteristic bacterial colonies. On June 8, about 20 per cent of the plants in this bed showed lesions on the lower leaves. After June 6, the disease gradually increased in number of plants infected and severity of attack. Many outer leaves and a few whole plants rotted off near the ground level. Plants with average or slight infection produced fine flower spikes. It was observed that occasionally of two plants springing from the same corm one would be infected, the other entirely free from infection. An adjoining bed of about 1,000 plants from newly purchased corms (varieties Augusta and Mrs. Francis King), growing on soil not recently, if ever, planted to gladioli, had on June 8, no indication of disease. Later in the season a few definite cases of the disease were found in this bed.

In the fall of 1921 the corms from both beds were dug and placed in storage. In the spring of 1922 they were planted in the plats used the two previous seasons. The early growth was normal but as the season advanced the disease appeared and increased in severity until by September practically all the plants were more or less affected. Out of about 1,400 (all that survived of the two lots) only 13 showed no outward sign of the disease. In 1923 the disease was again prevalent in the same plats of ground, planted with old and some new stock, but owing to cool weather in the early part of the season the disease was not evident until early July.

In an examination of a large planting in Michigan, where this disease had caused more or less trouble for the past 10 years, it was noted that the leaves of young stock were usually entirely free from the disease and that the older plants were affected in proportion to their age. This seems to indicate that a diseased plant carries the disease along from year to year, the disease increasing in severity until the plant succumbs. The grower stated that he believed this disease to be the eventual

[3] Reference is made by number (italic) to Literature cited, p. 117.



BACTERIUM MARGINATUM ON GLADIOLI PLATE 1

A.—Leaves entirely rotted at base. ×½.

B.—Outer leaves removed to show interior rot. ×½. A and B, natural infections collected and photographed July 21, 1920. Washington, D. C.

C.—Natural infection on leaf. ×4.

cause of the death of many of the older plants. There seems to be no generally used common name for this injury to the leaves. One grower suggests "neckrot" and as the "neck" of the plant is the most noticeably affected part this name seems appropriate.

The corms of plants affected with neckrot are not always diseased, but they often show very definite lesions on both the husks and the body of the corm. The infection may be so slight as to escape general notice, or so severe that the husks are practically destroyed and the corm covered with circular sunken spots.

In its early stages the husk lesion is pale yellow or pale brown in color, circular, oval or sometimes an elongated streak, with unbroken tissues. The color becomes darker, almost black; the husk splits longitudinally and also disintegrates at its base where it is attached to the corm (Pl. 3, A and B). The ragged margins have the texture and color of burned tissues.

Another type of husk lesion less likely to be observed is a thickened band of tissue on the inner side of the husks, near and surrounding the terminal bud (Pl. 3, H). Such lesions are found only by removing the husks. The tissues are several times the normal thickness, brittle or gritty like charcoal, and dark brown to black in color. Probably this represents the lower margin of a severe infection on the leafy part of the plant which has extended downward to the husk as a continuous lesion. Forty per cent of a lot of corms taken from storage in March had this type of lesion.

In most cases there is a direct relation between husk and corm lesions, the corm infection occurring just below the husk infection. Occasionally lesions are found under areas of healthy husk, but in these cases a careful examination shows some other lesion so situated that bacteria might pass from it under the husk to the place where the hidden lesion developed.

Corm lesions begin as definitely outlined, pale yellow water-soaked, circular spots. The epidermis shows no visible injury and all the tissues are firm. The color deepens, varying on corms of different colors, from light yellow-brown to dark brown or almost black. The epidermis remains intact over some lesions, but usually it becomes split or broken. Eventually the corm lesion becomes a shallow depression surrounded by a definite and somewhat elevated margin (Pl. 3, G). These depressions are usually only 2 to 6 mm. in diameter, but, when numerous, they coalesce and form large, irregular sunken areas. The pits do not extend deeply into the flesh of the corm and are rather easily removed, leaving a clean, saucer-shaped cavity lined with healthy cells.

Gladioli are subject to several diseases which produce corroded spots or pits on the surface of the corms, and although those caused by *Bacterium marginatum* are usually quite characteristic, it is realized that injuries due to other agents may produce lesions having a general appearance not unlike those which numerous isolation and inoculation experiments have proved to be caused by *Bacterium marginatum*.

The name scab is a good descriptive term for these lesions on the corms. This name occurs frequently in the literature of gladiolus disease and it is probable that in many instances the spots described were due to this bacterial infection. Some illustrations by Wallace (*11*) and others closely resemble the spots caused by *Bacterium marginatum*.

A rather striking phenomenon is the copious gummy exudate from the corm lesions. At first colorless, it changes to yellow-brown or dark brown, and when dry is brittle and shiny like varnish. The diseased husks are glued together and to the corm by this exudate and sometimes even the surrounding soil becomes impregnated with gum and forms a mass closely attached to the corm (Pl. 3, E and F). Just under each such mass of soil are the lesions from which the exudate flowed (Pl. 3, G).

It has not been determined whether this exudate is due to the action of *Bacterium marginatum* alone or aided by the various secondary organisms which attack the injured tissue. No gum exudes from leaf lesions or from quite young corm lesions. The exudate gives a positive reaction for sugar, which perhaps explains the presence of the many secondary organisms.

RELATION TO HOST TISSUE

Microscopic examination of sections of fresh and of stained material of leaf tissue shows that the bacteria are active only in the parenchyma. Bacteria have not been found in the vessels, but eventually in a diseased area the vessels are filled with a reddish-brown substance. The walls of vessels are browned and occasionally show browning even beyond the limits of the discolored parenchyma.

Bacteria enter through the stomata but it has not been proved that this is the only mode of invasion. Stomata

BACTERIUM MARGINATUM ON GLADIOLI PLATE 2

Natural infections. Collected and photographed June 20, 1922. Washington, D. C. +¼. Extensive rot at the base of *a* and *b* has caused these leaves to become dead and brown. The leaves just above *a* and *b* have isolated infection areas. (Red spider injury on older leaves.)

are present on both surfaces of the husks and on the epidermis of the corm. In leaf lesions the bacteria are most often found in small compact masses, sometimes only in a small area near the center. While numerous, they are not so abundant as in some other bacterial diseases. From fresh sections they ooze out only moderately and do not readily distribute in the mounting fluid. Capsules have been demonstrated on bacteria obtained directly from the plant and it is likely that this viscid covering tends to reduce the distribution of the bacteria both in the tissues and in the mounting fluids.

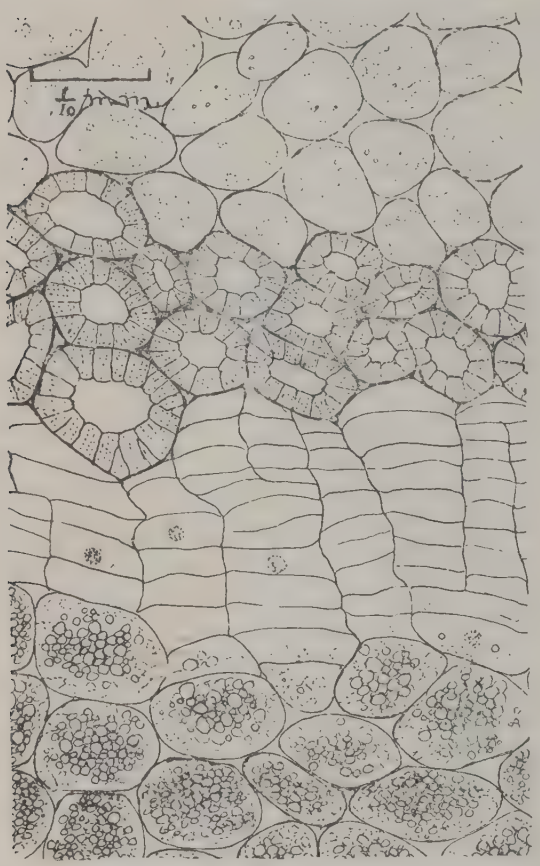


FIG. 1.—Camera lucida drawing of a section made through the base of a corm lesion. The healthy cells, full of starch, are separated from the diseased cells by cork and stone cells

Microscopic examination of the lesions removed from corms shows a wide layer of cork cells at the back, 10 to 12 cells deep, and just inside the cork are found several rows of stone cells (fig. 1). These protective layers of cork cells and stone cells are developed soon after infection occurs and evidently account for the small size and slight depth attained by the lesion. The ridge surrounding the lesion is formed by the out cropping of these cells. In the central part of the infected area are collapsed cells and a gummy substance, amber to brown in color, according to age. As in the leaf lesions, the bacteria are found in rather restricted areas near the center of the pit and not far below the epidermis.

These succulent corm lesions are subject to invasion by other organisms, both bacteria and fungi. Leaf and husk lesions are much less liable to secondary infections.

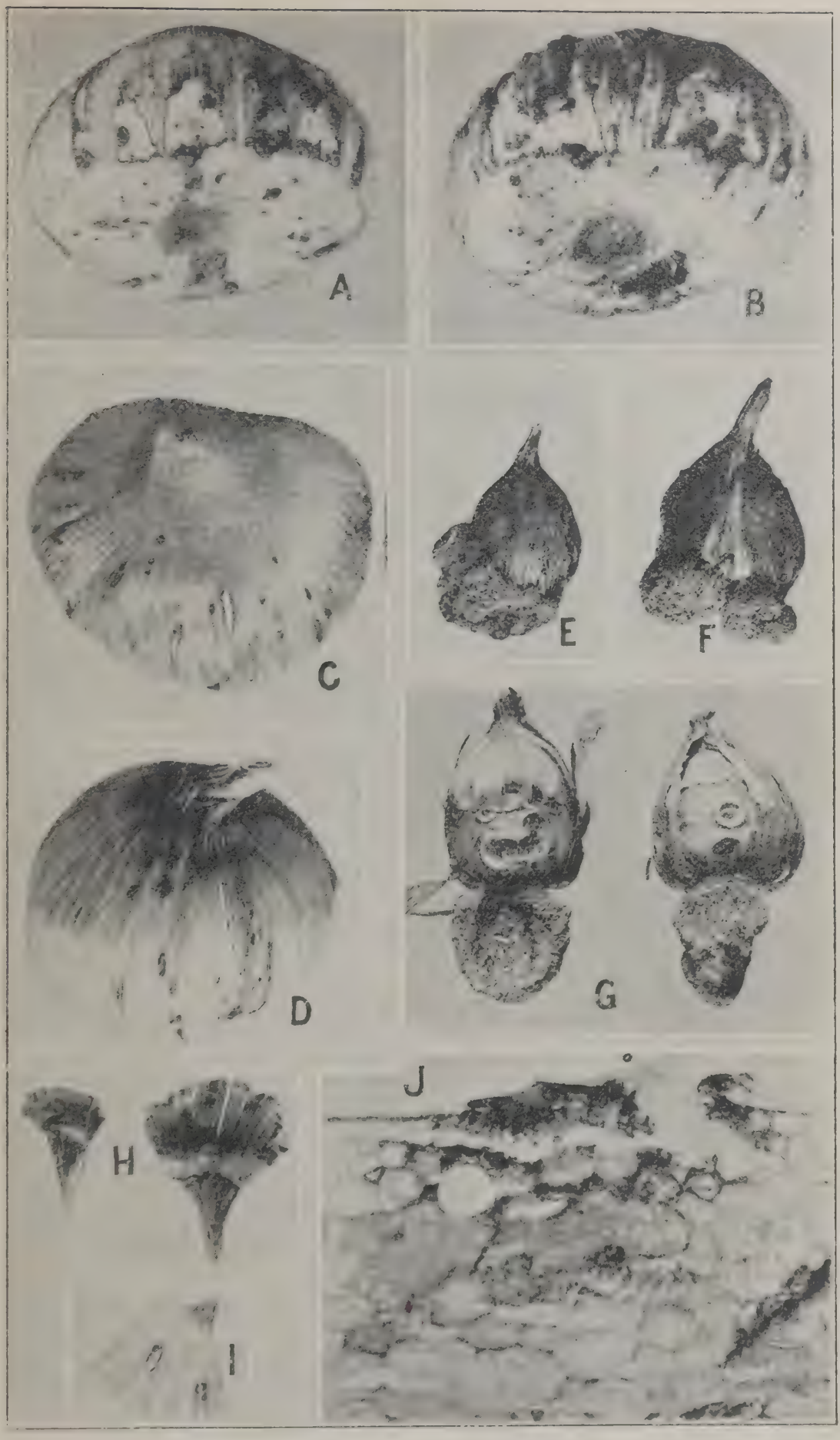
Growing corms which were inoculated with *Bacterium marginatum* had typical lesions 1 to 4 mm. in diameter 4 weeks later. The epidermis was intact and not visibly sunken though some of the cells just below the surface were brown and collapsed. Most of the interior cells were fairly normal in appearance and were still full of starch. The cork tissue at the back of the lesion was well developed but the stone tissue was barely discernible.

ISOLATION

The organism which causes this disease has been isolated repeatedly by means of agar poured plates from leaves, flower stalks, and corms, both from fresh and dry material, and also from the gummy exudate.

From either new or old infections on leaves and husks there is no difficulty in isolating *Bacterium marginatum*, but from any except recent corm lesions the plates are likely to have a discouraging number of various sorts of organisms. A corm spot that is still pale in color and with unbroken epidermis should be selected. From such spots with a sterilization of 2 to 3 minutes, absolutely pure cultures of *Bacterium marginatum* have been secured. The causal bacteria are present, in some at least, of the old lesions on corms, as has been proved by isolation experiments, but they do not often succeed in producing characteristic colonies in the isolation plates because of the great number and vigor of the secondary organisms that have invaded the lesions. Various sorts of bacterial colonies appeared in isolation plates made from old corm lesions, but only rarely were there any yellow colonies. If the yellow colonies produced looked at all like *Bacterium gummisudans*, which causes a leaf blight of gladioli (*3*), they were investigated more closely. None was ever found with the characters of *Bacterium gummisudans*, and inoculation experiments (see p. 167) further prove that *Bacterium gummisudans* is not connected with these corm lesions.

In a field where both *Bacterium marginatum* and *Bacterium gummisudans* occur, both diseases have quite often been found on the foliage of the



BACTERIUM MARGINATUM ON GLADIOLI PLATE 3

A and B.—Corms with husks badly infected and lesions on the body of the corm. ×7/8.

C and D.—Typical moderate infection of outer husks. ×7/8.

E and F.—Masses of soil held together and to the corms by the dried exudate from the lesions. ×3/4.

G.—Soil masses removed to expose the lesions. ×3/4.

H.—Husk lesions. Thick, crusty tissues on inner side of husks. ×3/4.

I.—Lesions on thin inner husk. ×3/4.

J.—Section through a corm lesion. The fine-grained cloudy part is full of bacteria ×about 160.

same plants. Because of this fact, particular care was taken to discover which, if either, was responsible for the corm spots.

Having also in mind the hard-rot disease of gladioli (4) caused by *Septoria gladioli* Passer and which produces on the corms lesions that sometimes resemble those caused by *Bacterium marginatum*, a careful watch was kept on the fungi isolated from diseased corms. No Septoria has been found among the various fungous growths secured. No Septoria disease has occurred on the gladioli, seedlings, or mature plants, used for experimental purposes in Washington and none has been observed in the various other plantings of gladioli from which diseased corms have been obtained.

Virulent colonies of *Bacterium marginatum* have been isolated in July from corms harvested the preceding October, thus definitely proving that the bacteria can survive on the stored corms from season to season.

For isolation from the gummy exudate from corm lesions, pieces of clean-looking gum were selected, rinsed quickly in sterile water, then dissolved in beef boullion. The plates from this unsterilized gum produced various growths, a fair proportion of which were *Bacterium marginatum*.

Infected leaves from which typical bacteria were isolated in August were kept in the laboratory all winter. The following March several efforts to isolate bacteria from lesions on these leaves resulted in failure.

Late in the fall some dry leaves were collected from the field. All parts were dark in color but the bacterial lesions could easily be distinguished. Examination showed that the bacteria were in small, isolated pockets in the lesions and under pressure oozed out in a thick mass. Typical *Bacterium marginatum* colonies were secured from this material.

Efforts were made to isolate the organism from plant parts that had remained in the field all winter. All the soft tissues had disintegrated, leaving only the flower stalks and the fibrovascular bundles of the leaves. Platings were made from black spots found on the stalks and from fibrous remains of leaves but among the great numbers of colonies that appeared in the plates there was none that resembled *Bacterium marginatum*.

No success has resulted from several attempts to isolate the organism from soil in which diseased plants had grown but the organism has been kept alive and virulent in both dry and moist soil in culture tubes for 11 months.

The pathogenicity of the bacteria isolated was proved in each case by successful inoculation and reisolation. Bacteria from leaf spots produced typical pits on corms and bacteria reisolated from these corms produced typical leaf infections. No difference in pathogenicity has been noted in the bacteria obtained from leaves, husks, or corms, from the different varieties of gladioli, or from different localities.[4]

INOCULATIONS

Numerous inoculation experiments have been made on sound gladioli, both in the greenhouse and outdoors. Bacteria from leaf lesions produced infection on both leaves and corms, and bacteria from corms as readily infected leaves as corms. Numerous isolations from leaves and corms have been tested and all seem essentially alike. In some of the early inoculations needle pricks were made, but it was soon found that it is not necessary to wound the epidermis. Bacteria from young agar cultures were diluted in sterile water and applied to the leaves either by spraying with an atomizer or spread on with a bit of cotton. Because of the extremely smooth surface and perpendicular position of gladiolus leaves it was often difficult to keep the inoculum on the leaf. Sometimes a few wet fibers of cotton were put on the inoculated areas. In others the smoothness of the leaf was somewhat reduced by rubbing with a bit of cotton. In dry weather, out of doors, a layer of wet cotton was placed over the inoculated area.

Under average summer conditions in the field the infections show definitely on the leaves in from 7 to 12 days after inoculation as tiny, isolated spots of bright reddish-brown color, usually very numerous in the area inoculated. These by enlargement and coalescence form the larger lesions (Pl. 1).

In the greenhouse, if the inoculated plants were kept for the first 24 hours

[4] Dr. Ivan C. Jagger isolated *Bacterium marginatum* from gladioli grown in Florida from corms obtained from a New York firm. The disease appeared during the summer rainy season. Doctor Jagger states:

During four years spent largely in Florida I examined several small plantings of gladioli, and invariably found no leaf spots. Generally gladioli are planted out in the late fall or early winter so that they come into bloom rather early in the spring, when the weather is quite generally dry and unfavorable for leaf blights. My planting, which developed bacterial leaf spot, was made much later in the season than is favorable for best results.

in a moist case, the infection showed plainly in from 3 to 5 days. If moist conditions were continued, a rather extensive soft rot occurred, the tissues becoming gray-green and water-soaked.

Sterilized soil, sterile water, and a pure culture of the bacteria were mixed, then spattered over the leaves of healthy plants. No effort was made to wound the epidermis. This inoculum was kept moist for 12 hours by placing the plants in a moist case. The plants were then returned to the open bench where the usual watering quickly washed off the soil particles. Tiny spots due to the bacteria were noted on the sixth day and by the ninth day the largest spots were 2 mm. in diameter. There were 200 or more spots on the inoculated side of the leaves. The opposite side where no inoculated soil had been placed had a few scattered infections. The spots enlarged, coalesced, and caused the death of the inoculated leaves.

Soil from outdoor beds where diseased gladioli had grown the previous season has been used to inoculate gladioli. The soil was moistened with sterile water and spread in a thick layer over the lower leaf parts of vigorous greenhouse plants or by placing on the leaves a layer of cotton soaked with the soil and water mixture. The moist condition of the soil inoculum was retained for several days by keeping the plants in a moist chamber. This experiment was tried in May, 1922, in April, 1923, and in March, 1924. No infections resulted from any of these inoculations.

Garden soil from plats where the gladiolus disease had occurred for three seasons was brought into the greenhouse in January and healthy gladiolus corms were planted in it. These corms had the husks removed and the surface sterilized by a 15-minute treatment in a mercuric chloride, 1–1000, solution. Examination in June showed definite and typical spots on the new corms and isolation plates from both husk and corm spots gave pure cultures of *Bacterium marginatum*. Control plants in sterile soil showed no signs of infection.

On corms the lesions produced by inoculation were slower in growth than those on leaves. Development may be more rapid under more favorable conditions. The quickest results were secured on young, growing corms by removing enough soil to expose most of the corm but without disturbing any of the roots. The soil particles were washed off, bits of the husk were loosened and lifted so that bacteria could be placed directly on the uninjured surface of the corm. The husks were replaced and the corm again covered with the soil. Typical brown pits, 1 to 4 mm. in diameter, were found 4 weeks after such inoculations and pure cultures of *Bacterium marginatum* secured from them.

In other corm inoculations the bacteria were placed on the husks or the soil was thoroughly wet several times during the growing season with water containing bacteria. Both of these methods gave infections but they were slower in developing than the preceding.

That mature corms are less subject to infection than young, growing corms was shown in the following experiment. Seventy healthy, husked, and sterilized corms were planted in pots in sterilized soil. Most of these were inoculated with pure cultures of *Bacterium marginatum*, others with crushed lesions from diseased corms. These dry lesions taken from corms harvested several months previously were pounded in a mortar and softened with water. In each case the inoculum was poured over the uninjured corms when planted. The inoculation was repeated several times during the early growing period by pushing the soil away and pouring the inoculum over the corms. When harvested only 13 of the 70 old corms showed signs of infection. In 31 pots of this lot of 70 the inoculation was resumed during the growth of the new corms and 27 of the new corms (of the lot of 31) had typical husk and corm lesions from which the bacteria were isolated and their pathogenicity proved.

Fifty-two healthy, husked, and sterilized corms were planted in pots in sterile soil. Pure cultures of *Bacterium gummisudans* (*3*), also fresh leaf lesions caused by that organism, were used to inoculate these corms. The inoculum was applied and repeated at intervals as in the preceding experiment. When mature a few typical husk spots and corm pits were found on three of the new corms. Isolation plates were made from all three and in each case *Bacterium marginatum* was secured but not a single colony of *Bacterium gummisudans*. These 52 plants were grown in the same greenhouse and at the same time as those inoculated with *Bacterium marginatum*. They were on separate benches and care was exercised to avoid contaminations. It is not known just how these three corms became infected with *Bacterium marginatum*.

Twelve healthy corms, husked, sterilized, and planted in sterile soil were

inoculated with cultures of various fungi that had appeared in isolation plates inoculated from corm lesions. The inoculations were repeated, as in the preceding experiments, but neither the old nor the new corms became infected.

Introducing *Bacterium marginatum* into wounds made in mature corms did not produce any rot. This was tried three times with different degrees of heat and moisture, using varieti s known to be susceptible. There wa no reaction beyond a slight browning and the formation of an imperfect layer of cork. These corms were then planted and produced normal plants. Tests showed the juices of these gladiolus corms to be very acid. It is possible that the bacteria coming into direct contact with large amounts of the acid juice from the wounded tissues were not able to grow.

Potato tubers were copiously inoculated with this organism but no trace of rot resulted.

MORPHOLOGY

Obtained directly from the host the organism is at first sluggishly motile but after a few minutes it becomes more active. Staining with carbol fuchsin shows rods of varying sizes, 0.8 to 1.8 by 0.5 to 0.6μ. Many are so short as to resemble cocci. The rods are rounded at the ends and occur singly and in pairs.

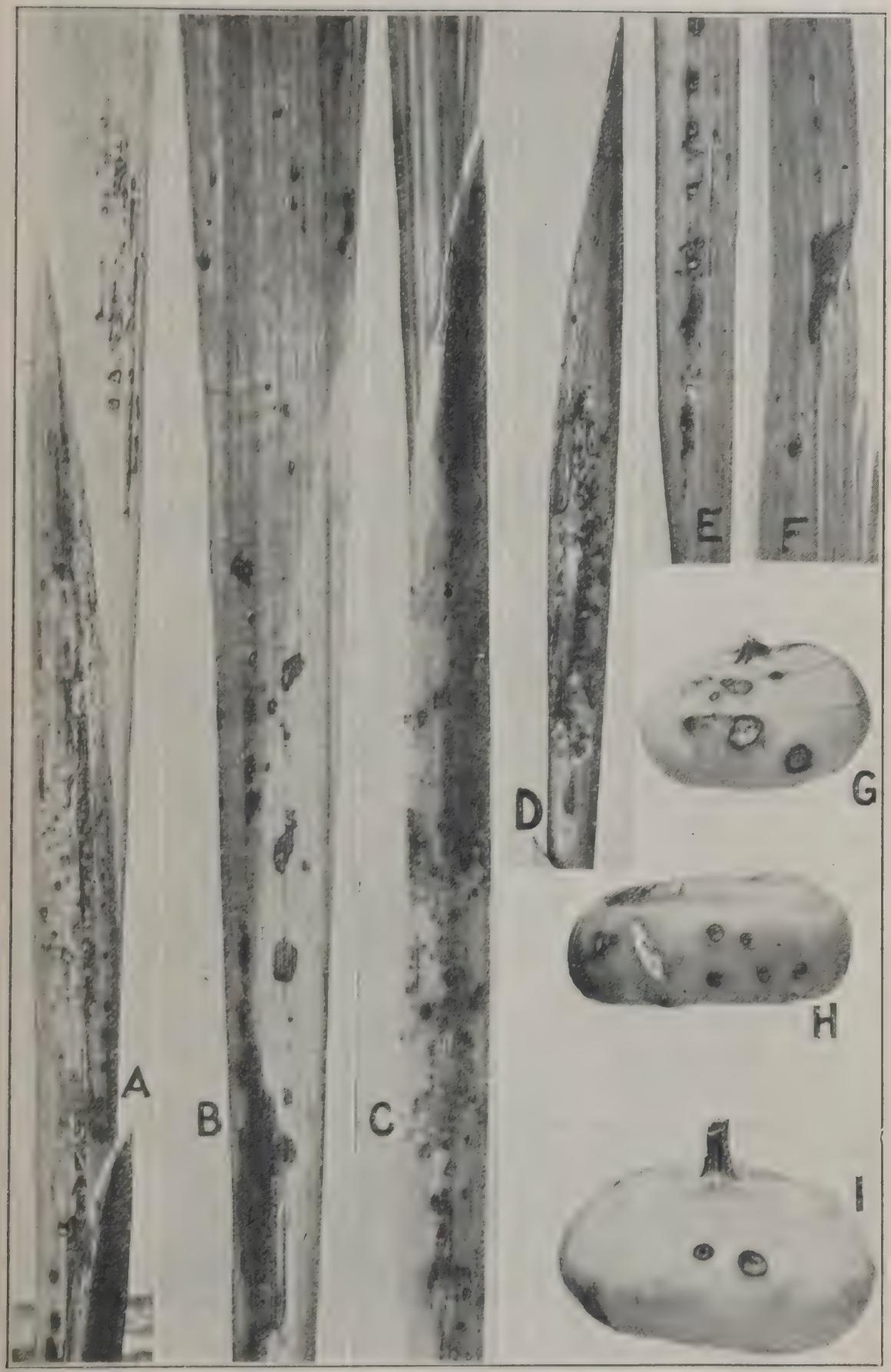
From 3-day-old cultures of peptone-beef bouillon the rods stained with carbol fuchsin measure 0.9 to 1.8 by 0.5 to 0.6μ. Longer rods are found, but these usually show division lines. The bacteria stain well with all the usual stains. Capsules are present on bacteria obtained directly from the host and are also formed in beef media, and in Thaxter's potato agar (Ribbert's capsule stain was used). No spores have been found. The organism is motile by means of 1 to 4 bipolar flagella. The flagella are mostly found at one end only. However, careful search shows occasional rods with flagella at both ends; a single flagellum at one pole and one to two, rarely three flagella at the opposite pole. In the mounts examined the rods having four flagella at one pole have none at the opposite pole (Pl. 5, I). The flagella are 3 to 8μ long. These were stained by Casares-Gil's method. Gram-negative. Not acid fast. Long chains develop in 2 and 3 per cent salt-beef bouillons, short chains in the usual beef media. Involution forms as very slender granular rods were found in beef bouillon cultures grown at 39° C. In potato agar plus dextrose the bacteria are usually large, some as wide as 0.8μ, and rather granular. In 1 to 4 per cent dextrose water cultures the growth s scanty and numerous, myceliumlike forms are produced.

CULTURAL CHARACTERS [5]

AGAR POURED PLATES.—On peptone-beef agar, reaction +10 to +14, colonies appear in 18 to 24 hours and under favorable conditions are often 10 to 12 mm. in diameter at the end of 5 days. Colonies are at first circular, smooth, shining, almost hemispherical, usually amorphous and opaque, sometimes thinner, translucent and with concentric striations. Margins entire. In 1 to 3 days a marginal growth develops, which increases from a thin, narrow, barely visible film (Pl. 5, A) into a well-defined border of considerable width and thickness. Average colonies 4 to 5 days old have centers 2 to 3 mm. in diameter, surrounded by borders 2 to 4 mm. wide. (Pl. 5, B, C and G). Well isolated colonies sometimes produce borders 7 to 8 mm. in width (Pl. 5, D). The border, at first extremely and uniformily thin, becomes thicker and contoured by the development of short, more or less irregular, radiating ridges. In 8 to 14 days the border is about as thick as the central area which remains smooth and does not increase appreciably in diameter (Pl. 5, E). The appearance and development of this outer margin is greatly influenced by the temperature and the degree of isolation. The growth is white, soft, and viscid in consistency. Buried colonies are round to oval, occasionally triangular, outlines smooth, opaque becoming translucent. In thickly sown plates the characteristic marginal growth may be so delicate as to escape casual observation and sometimes it is entirely lacking.

AGAR STABS.—In peptone-beef agar growth is good on the surface, uniformly thick, smooth, white, opaque to translucent. The stab is visible for a

[5] Cultures were grown at room temperature, approximately 25° C., unless otherwise specified.
Beef infusion was used for all the beef media.
Difco peptone and Eimer and Amend agar were used in the media.
For the media used for the optimum reaction for growth three sets of determinations were made. First, it was titrated and the Fuller scale readings obtained, then PH determinations were made colorimetrically, according to the Clark and Lubs' method, and electrometrically.
"Color Standards and Color Nomenclature": Robert Ridgway, used in describing color in cultures (7).



BACTERIUM MARGINATUM ON GLADIOLI PLATE 4

Leaves and corms inoculated with pure cultures of *Bact. marginatum*. All natural size.

A.—Inoculated with bacteria from gummy exudate. Photo 20 days after inoculation.

B to E.—Inoculated with bacteria from corm lesions. B, 10 days, C, 9 days after inoculation.

F.—Lesions on upper part of leaf, 11 days after inoculation.

G, H, I.—Healthy, growing corms inoculated in May by adding bacteria to the soil. Dug a month later and pure cultures of *Bact. marginatum* isolated from pits on each corm.

few days as a finely granular streak, then disappears.

AGAR SLANTS.—On peptone-beef agar growth is smooth to slightly pitted. Margins definite, entire. Transparent to translucent. White, very viscid. On 2 per cent agar the growth is somewhat restricted. On 1 per cent agar the growth quickly spreads over the surface and forms a pellicle over the water in the V and a white, fine grained sediment is deposited.

On beef agar there is often a lack of uniformity in the interior structure of the growth, some areas being quite translucent, others opaque. This gives a mottled appearance to the growth.

BEEF BOUILLON.—In peptone-beef bouillon+14 at 24° C. there is thin clouding and a thin pellicle in 18 hours. Clouding is best at the surface and never becomes heavy. Pellicles are white, membranous, and viscid, falling when even slightly disturbed and another pellicle forming promptly. As many as five definite pellicles have formed in one culture tube. Sediment consists of a scanty amount of white substance, small crystals, and fallen pellicles. Pellicles eventually disintegrate and form a viscid, translucent mass. The medium also becomes viscid.

GELATIN PLATES.—In +10 peptone-beef gelatin at 18° to 20° C. the colonies grow slowly. They have the same character in regard to center and border as colonies in agar plates. The borders remain thin and narrow and are slightly lobed at the margin. Liquefaction was noted first after 5 to 7 days as a narrow rim about the colony. The margin of liquefaction is smooth and definite. Eventually the colony floats in a clear liquid.

GELATIN STABS.—At 18° to 20° C. in +10 gelatin liquefaction becomes visible in 48 hours and proceeds at a moderate rate. In 5 to 6 days the liquefied layer is 5 to 7 mm. deep. There is a thin, white pellicle, thinly clouded liquid and a small amount of white sediment. Four to six weeks are required for complete liquefaction of tubes containing 10 cc. of gelatin.

BLOOD SERUM.—On Loeffler's blood serum at room temperature (25° to 27° C.) growth is abundant, smooth, white, opaque. The medium becomes translucent, light amber in color, and the consisteney of soft jelly.

POTATO CYLINDERS.—Growth on steamed potato is scanty. A very thin, white growth over the potato and slight white deposits along margins where the potato touches the tube are the only indications of growth. The water remains clear, sometimes slightly browned. The potato discolors slightly. The diastasic action is feeble.

POTATO AGAR.—Potato broth with agar. Growth on this medium is limited. Thin, white, becoming dull white. No change in the color of the medium.

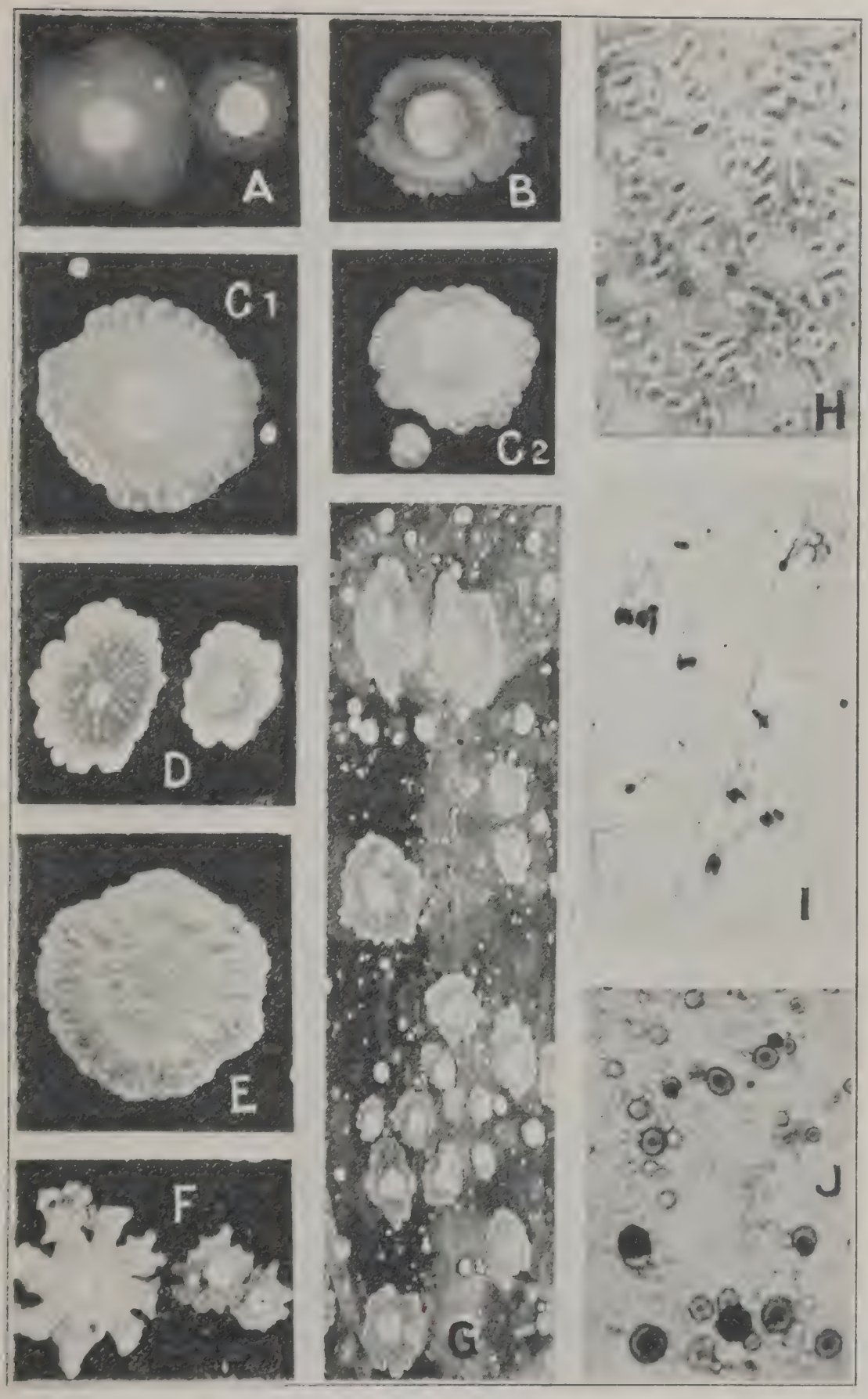
POTATO AGAR WITH DEXTROSE [6].—Colonies in plates are at first smooth, shiny, hemispherical, like tiny drops of boiled starch. In 2 to 3 days the surface is finely wrinkled or coarsely pitted, and a trace of brown color has appeared. In 5 to 6 days the colonies are rather umbonate and brown at the margins. In 10 to 12 days the whole colony is brown and widely separated colonies have developed a new, thin, cream-colored marginal growth. Colonies in thickly sown plates are less likely to turn brown than well isolated colonies.

On slants and stabs the growth is first white, opaque, and slightly contoured. Later the growth is very abundant and the surface becomes smooth and shining. The color of the growth in most isolations becomes tawny to russet brown, the color usually showing first at points where the growth touches the wall of the tube. On soft, moist agars the growth is often quite smooth from the beginning. The growth is butyrous in consisteney. Some isolations produce a dark-brown growth, others become only slightly brown. Microscopic examination of the brown growth shows the color to be due partly to brown bacteria which vary in size from small coccilike forms to abnormally large rods; and partly to brown, spherical bodies (Pl. 5, *J*) the character of which is yet to be determined. They may be degenerate bacteria, or a substance formed by bacterial action.

The brown bacteria and the brown spheres occur together in pseudozoogloeal masses, the surrounding growth consisting of normal, hyaline bacteria.

There is also produced a definite change in the medium as evidenced by an area of increased translucency bordering the brown growth. This forms a halo about plate colonies, rapidly spreading out until all the agar in the plate is changed. In stab and slant tube cultures a deep layer

[6] A modified Thaxter's potato agar. Potato 300 grams, sliced and steamed, in 1,000 cc. of distilled water, strained, 1½ per cent agar added, steamed again, filtered, 2 per cent dextrose added, tubed, and sterilized.



BACTERIUM MARGINATUM ON GLADIOLI PLATE 5

A.—Colonies on beef agar, 2 days old. ×3
B.—Colony on beef agar, 3 days old. ×3.
C.—Colonies on beef agar, 5 days old. ×3
D.—Colonies on beef agar, 6 days old. ×2.
E.—Colony on beef agar, 7 days old. ×3.
F.—Colonies 3 days old. Plated from beef bouillon that clouded at 40° C. ×4
G.—Colonies 5 days old. Oblique lighting to show elevation. ×1¾.
H.—Capsules, 2-day beef agar culture. Ribbert's Dahlia stain. ×2000.
I.—Flagella. Casares-Gil stain. ×1,500.
J.—Brown spheres formed in potato agar plus 2 per cent dextrose. ×500.

below the growth becomes more translucent. Tests show that the sugar has disappeared from this changed part of the medium but that the starch content is practically the same as in the controls.

A very characteristic odor develops in cultures on this medium. It suggests ammonia, also hydrogen sulphide, sauerkraut and fermenting fruits. Cultures kept on artificial media for long periods produce less odor and less brown color than recently isolated cultures.

STARCH AGAR.—To ordinary peptone-beef agar 1 per cent of potato starch was added. Streaks were made on this medium and colonies also were grown by the usual poured-plate method. Growth was abundant and the marginal growth was unusually wide, 5 to 20 mm., and thin on both the streaks and colonies. After 10 days the plates were flooded with iodine potassium iodide. Seven isolations including the one selected as the type showed no diastasic action on the starch. One other isolation gave a very slight reaction.

A medium of potato starch, dextrose and agar was tried but the organism produced on this very scanty growth. The medium just below the growth was tested and gave as strong a starch reaction as the control.

MILK.—Inoculated milk forms a smooth, soft coagulum in 3 to 4 days. Clearing begins promptly and is complete within 2 weeks at temperatures 30° to 33° C. (At temperatures 26° to 29° the digestion is somewhat slower.) The medium becomes pale yellow, translucent and very viscid. After some weeks the color deepens to light brown or deep cream. Reaction is at first acid, then alkaline.

LITMUS MILK.—There is a slight, fugitive reddening which is likely to be overlooked unless cultures are closely watched. Reduction begins promptly and is complete in from 6 to 8 days. In 2 weeks or less the blue color begins to return and in 4 or 5 weeks the cultures are dark blue.

Some puzzling crystals formed in milk cultures. In general they may be described as brittle, hollow spheres 1 to 1.5 mm. in diameter; fairly smooth on the surface but not shining. A few were found hanging from the pelliclelike surface growth but most were on the surface of the sediment. Under microscopic examination the wall appears to be composed of minute crystals and some amorphous substance. They resist considerable pressure. If cut, the edges are fairly smooth. The interior cavity has a diameter about one-half that of the whole sphere. Water does not dissolve these crystals, neither does 10 per cent KOH so it would seem that they are not composed of sugar or casein. They dissolve in acetic acid, dilute hydrocholoric acid, and sulphuric acid. Alcohol, xylol, ether, acetone, chloroform, and ammonia had no effect.

LITMUS AGARS WITH SUGAR.—Litmus agar with 1 per cent peptone and 1 per cent of dextrose, lactose, saccharose, maltose, galactose, mannit, or glycerin was used. Reddening occurred within 24 hours in the dextrose and galactose. Lactose, mannit, maltose, saccharose, and glycerin showed slight reddening in 3 days.

FERMENTATION TUBES.—Fermentation tubes containing 2 per cent Difco peptone and 2 per cent dextrose, saccharose, lactose, galactose, maltose, mannit, or glycerin produced abundant clouding and a pellicle in the open end. Dextrose and galactose gave most growth and in these there occurred a faint clouding after 12 days in the closed ends. No gas formed.

Fermentation tubes containing sterile milk produced no gas. The milk cleared rapidly in the open end, followed by slow clearing in the closed end. The reaction of the cleared milk was alkaline.

Nitrate bouillon in fermentation tubes clouded well and formed a pellicle in the open end within 2 days, and a faint clouding in the closed end was observed on the fifth day in one set of cultures and on the tenth day in another set. Ammonia was produced. Nitrates were not reduced. No gas was produced.

TOLERATION OF SODIUM CHLORIDE.—In peptone-beef bouillon containing 2 per cent sodium chloride, the growth is practically normal. Growth becomes less in 3 per cent, very scanty in 3½ per cent, and in 4 per cent no growth occurred.

USCHINSKY'S SOLUTION.—Thin uniform clouding occurs in 24 hours. A pellicle forms. There is no color reaction except a trace of green in some cultures after 8 weeks. The medium becomes as viscid as egg albumen and with age and evaporation it is almost like rubber.

FERMI'S SOLUTION.—Growth is better in Fermi's solution than in Uschinsky's. Thin, membranous pellicles form repeatedly. As many as nine pellicles have formed in one culture tube. After 3 to 4 weeks the medium is pale yellowish green. Not viscid.

COHN'S SOLUTION.—Growth is slow at first but after a few days is about

equal to that in Uschinsky's solution or beef bouillon. Numerous crystals form in the pellicle, in fact the weight of the crystals causes the pellicle to fall. Fallen pellicles and crystals form the sediment. The pellicles and sediment are slimy to viscid. In most tests the sediment becomes yellowish. One set of cultures developed a pale but clear orange-yellow color in the pellicles, crystals, and sediment.

NITRATE BOUILLON.—There is good growth in this medium, but nitrates are not reduced.

INDOL.—In Dunham's solution, in 1 per cent and in 2 per cent peptone water a slight amount of indol was produced.

AMMONIA.—Tests were positive for ammonia in cultures grown in 2 per cent peptone water, milk, beef bouillon, and other media.

HYDROGEN SULPHIDE.—Produced only in very slight amounts.

AEROBISM.—Shake cultures of agar show growth only at the surface. No growth occurs at the lower end of stab cultures in agar or gelatin. Plate and tube cultures made no growth in an atmosphere deprived of oxygen.

OPTIMUM REACTION FOR GROWTH IN BOUILLON.—The organism grows best in peptone-beef bouillon titrating +8 to +12. Sodium hydrate was used as the alkali and hydrochloric as the acid. It grew well in various acid reactions up to +40. Growth occurred in +43 in 48 hours and in +45 within 6 days in one case and within 10 days in another. No growth developed in medium more acid than +45. The range of growth was more limited on the alkaline side. The amount of growth gradually lessened from +10. In −4 to −5 clouding was evident within 24 hours. In −6, −7, and −8 clouding was not observed until the second day. In −9, no growth. In several cases clouding was observed on the fifth day in −9, but here it was assumed that the medium had become more acid in the interval.

In addition to the titration by Fuller's method with phenolphthalein used as the indicator, colorimetric determinations (Clark and Lubs' series) were usually made and in some cases determinations by the electrometric method (Table I).

In bouillon made with beef extract and titrating +30 the organism made no growth. With this mediun titrating from +3 to +25 the organism grew within 24 hours.

TOLERATION OF ORGANIC ACIDS.—Peptone-beef bouillon +12 containing malic, citric, oxalic, and tartaric acids in amounts to make 0.1, 0.2, and 0.3 per cents were inoculated from 3-day-old beef bouillon cultures. The organism grew well in the 0.1 and 0.2 per cent of malic, citric, and tartaric acids. In 0.1 per cent oxalic acid growth occurred only as a surface pellicle. No growth occurred in 0.2 per cent oxalic, or in any of the 0.3 per cents acids.

TABLE I.—*Reactions of Bacterium marginatum to acid and to alkali in beef bouillon (+ indicates growth; −, no growth)*

Fuller	Colorimetric	Electrometric	24 hours	48 hours	5 days
	P_H	P_H			
−9	9.2	8.96	−	−	−
−9	9.1	8.63	−	−	+
−8	8.8		−	+	+ very faint
−7	8.8	8.5	−	+	+
−6.5	9.0	8.59	−	+	+ faint
−5	8.7		+ very faint		+ faint
−4	8.6		+ faint		+
−2	8.3		+ faint		+
0	8.2		+ faint		++
+5	7.9	7.7	+	++	+++ abundant
+8	7.2		++ moderate	++	+++ abundant
+10	7.1		++ moderate	++	+++ abundant
+12	7.0	6.89	++ moderate	++	+++ abundant
+15	6.7		+		++
+20	6.6	6.55	+		++
+25	6.4	6.26	+ faint		++
+33	5.3		± faint		++
+35	5.2	4.87	+ faint		++
+39	4.8	4.6	+ very faint		++
+39			−	+	++
+41			−	+	++
+43			−	+	++
+44(?)	4.6	4.62	−	+	+
+45(?)	4.6	4.58	−	−	−
−46(?)	4.6	4.61	−	−	−

Titration of these media showed that the 0.1 per cent acid media ranged from +20 to +26, the 0.2 per cent from +42 to +43, and the 0.3 per cent from +63 to +78.

TEMPERATURE RELATIONS.—In peptone-beef bouillon the optimum temperature for growth is about 32° C. Clouding occurs more promptly at 34° to 35° than at 30° to 32°, but after 24 hours growth is better at the lower temperature.

The minimum temperature for growth is at 8° to 9° C. The organism remained alive for 4 months, and probably longer, at 1° to 2°. The maximum temperature is 40°. Various trials indicate a thermal death point at 52° to 53°. Occasional cultures have given growth after a 10-minute exposure to 54°.

FREEZING.—The freezing for 1 hour of 24-hour-old beef bouillon cultures reduced the number of bacteria 40 to 50 per cent.

DESICCATION.—Drops of beef bouillon cultures 18 to 48 hours old were transferred to sterile cover glasses and dried in sterile Petri dishes. The results indicate that the younger cultures are less resistant than older. This may be due to the fact that the older cultures are already somewhat viscid, do not spread so well, and probably retain moisture longer than the young cultures. Young cultures resisted drying from 4 to 5 days. Older cultures gave growth up to 14 days.

EFFECT OF SUNLIGHT.—Beef-peptone agar plates, thinly sown, were exposed on ice to sunlight in July at 11 a. m. Ten minutes' exposure killed 50 to 70 per cent and 20 minutes' exposure killed practically all the bacteria.

VITALITY.—At Washington room temperatures the bacteria in culture media die in 7 to 9 months. This may be due partly to drying of the media. At lower temperatures (14–18° C.) cultures live more than a year but the pathogenicity is reduced by age. Some young cultures were mixed with sterile soil which was then placed at 1–2° for 4 months, then at 8–9° for 3 weeks. From a bit of this soil the bacteria were isolated in great numbers. These tubes then remained in a closet at room temperature and the bacteria were alive and virulent 11 months after being put in the tubes. After 15 months isolations showed the bacteria still very much alive but no pathogenic test was made. No growth was secured from lesions on leaves kept at room temperature for 7 months.

VARIATIONS.—Cultural studies and infection experiments have proved that the various isolations are similar in their essential characteristics, though not absolutely alike. One isolation from plants grown in Virginia liquefies gelatin more rapidly than the one selected as the type. Another from Michigan produces more abundant growth on artificial media. Some isolations are less viscid than others and occasional sets of beef bouillon cultures never become viscid. The bacteria isolated in 1913 browned beef media. Later isolations have not discolored beef media but some of them have slightly browned potato agar plus dextrose. The brown growth on potato agar plus dextrose occurs in most but not all of the isolations.

GROUP NUMBERS.—The group number, according to the descriptive chart (1907) of the Society of American Bacteriologists, is 211.2222022. According to the 1920 chart it is 5322–31130–1222.

TECHNICAL DESCRIPTION

Bacterium marginatum, n. sp.

Cylindrical rods rounded at ends, occurring singly and in pairs; 0.8 to 1.8 by 0.5 to 0.6μ; aerobic; motile by means of one to four bipolar flagella; no spores; capsules present; stains readily but often shows granular structure; Gram-negative; not acid fast.

On beef agar the colonies are white, circular, becoming more or less irregularly lobed; center smooth, almost hemispherical, surrounded by a wide border which is thin at first becoming thick and contoured.

Liquefies gelatin and softens blood serum; produces slight amount of acid in milk, then becomes alkaline; digests casein; produces acid in cultures with various sugars, especially in galactose and dextrose. Nitrates are not reduced. No gas is produced. Slight amounts of indol, hydrogen sulphide, and ammonia are produced. Grows well in Uschinsky's, Fermi's, and in Cohn's solutions. Viscid in most media. Not viscid in Fermi. Temperature for growth, maximum 40° C., minimum 8° to 9°, optimum 30 to 32°. Thermal death point about 53°. Remains alive at temperatures below 9° for 4 months or more; not particularly sensitive to drying; freezing for 1 hour killed 40 to 50 per cent; sunlight for 10 minutes killed 50 to 70 per cent.

Pathogenic on gladiolus leaves and corms, producing reddish brown lesions, circular to elongated on the leaves, and circular, shallow brown pits on the corms.

Specimens of the disease on leaves and corms have been deposited in the

herbarium of the Bureau of Plant Industry, United States Department of Agriculture.

SUSCEPTIBILITY

This disease has been observed on practically all the varieties of the gladiolus commonly grown by florists but most of the inoculation tests and close observations have been on the varieties America and Mrs. Francis King.

OVERWINTERING AND DISSEMINATION

It has been demonstrated that the organism may be carried over from season to season on the corms. The causal organism has been found in a living and virulent condition on corms, stored under usual conditions, 9 months after being harvested. Corms as badly marred as those shown in Plate 3, with the husks so disintegrated that the pitted body of the corm is exposed, would probably be discarded by most growers. Other corms having small, inconspicuous spots on the outer husks might pass as healthy. These spots may or may not extend through the several layers of husks to the corm.

Infected husks are probably as great, if not a greater, source of infection than infected corms, for the reason that at planting time or later in cultivating, broken or loose pieces becoming incorporated with the soil, may come in direct contact with the plants.

Great numbers of the parasite are often found on the husk parts through which the growing bud must push and some of the infecting material, carried along with the growing leaves, may eventually succeed in producing leaf infections which become foci for other infections. Meanwhile on the parent corm the husks are disintegrating and the body of the corm is changing to a shrunken mass on which the old pits are still distinguishable but not increased in size or depth.

Observation of many plants indicates that the new corm, though in such intimate contact, is not ordinarily directly infected by the diseased parent corm. Many healthy plants have been grown in the greenhouse from diseased corms. The husks were removed and the corms planted rather deeply in clean sandy soil. These plants were not repotted nor was the soil disturbed in any way. In practically every case healthy plants and clean, new corms were produced. Similar corms planted outdoors in a bed where the disease had occurred for several years and cultivated in the usual way, invariably became infected. It was observed that the loose soil was dashed on the plants by every rainstorm and considerable soil left sticking to the lower leaf parts and that infection occurred most abundantly on the parts spattered with soil. This, as well as some other experiments, indicates that the infection came from infected soil.

Inoculation experiments have shown that the corms are most easily infected when the husks are young and still full of moisture. Unless the bacteria come in contact with the husks during this susceptible period the parasite seems unable to penetrate deeply enough to reach the body of the corm.

Favorable temperature and moisture of the soil are also doubtless factors in the initial infection and in the continued activity of the bacteria. It has been noted that plants in the moister parts of fields and beds have heavier infection than surrounding plants in better drained soil.

The part played by insect transmission of the disease also needs to be considered. In a few cases of corms planted in unsterilized garden soil, mites have been found in connection with the lesions of the husks and corms. The mites had made perforations in the dark husk spots and small burrows in the corm spots. Except for the holes these lesions were typical for *Bacterium marginatum* and this organism was isolated from both husk and corm spots that definitely showed mite attacks. If mites are able to penetrate normal gladiolus tissues, they probably aid in distributing the infection.

DISINFECTION EXPERIMENTS

Disinfection experiments have not been extensive but it is believed that corms can be treated so as practically to control the disease.

The following experiment was made, using badly pitted corms, variety Mrs. Francis King:

The corms were first soaked for 15 minutes in water, then the water was poured off and the corms were protected so as to retain moisture for several hours. This process softened the hardened exudate, which otherwise might have interfered with the action of the disinfectant. Four lots were then treated for one-half hour as follows—

(*a*) Mercuric chloride, 1–1000.
(*b*) Copper sulphate, 1 ounce to 1 gallon of water.
(*c*) Formalin, 1–40.
(*d*) Formalin, 1–80.

After this one-half hour treatment the solutions were poured off and part of the corms planted at once in sterile soil. The others were allowed to dry.

When the corms had dried, lesions were taken from all the lots and isolation plates made without any further sterilization, but each lesion was washed through three changes of sterile water to remove any disinfectant that might be on the surface.

Bacterium marginatum was secured from husks of the corms treated with mercuric chloride, but considering the heavy inoculation of the plates, there were not many viable bacteria in these husks. No *Bacterium marginatum* was obtained from the husks of the other three lots or from corm spots of any of the four. A small number of bacteria and fungi of several sorts appeared in the plates. In general, there was a striking reduction in the number of organisms.

Three weeks after treatment the rest of the corms were planted in pots of sterile soil. All the corms produced healthy, vigorous plants. Six months later when they were dug the new corms and cormlets were free from spots, except a few in the lot treated with copper sulphate, which had slight but definite husk lesions.

In another experiment typically pitted corms of the varieties Mrs. Frank Pendleton, Mrs. Francis King, Schwaben, America, and a few of unknown variety, were treated for 1½ hours in formalin 1–60, then planted while still damp in clean soil in pots. There were 80 corms in this lot and the husks were not removed. All grew well and produced vigorous plants. Four and a half months later a few were blooming and in most the new corm was fairly well developed. No sign of infection was found on leaf parts or on corms.

Sixty healthy corms were treated the same as the pitted corms. Fifty were planted in sterile soil and ten in soil taken from a bed where the bacterial disease had occurred the previous season. Those in sterile soil produced clean plants and corms. Those in the infected garden soil had no infection on the parts above ground, but the "neck" and new corm of all showed typical spots after 5 months' growth and isolation plates produced typical colonies of *Bacterium marginatum*. Special effort was made to keep the soil surface firm and there was practically no soiling of the lower leaves.

OTHER HOSTS

On iris a very weak infection occurred if the inoculated areas were kept moist for several days. The spots caused by the bacteria were reddish-brown surrounded by a water-soaked area.

Calla lily leaves and petioles were inoculated but never showed any infection.

OTHER BACTERIAL DISEASES OF THE GLADIOLUS

In 1913 G. Severini (*10*) published descriptions of two bacterial diseases of gladioli. Both are soft rots attacking chiefly the underground parts of the plants. Neither of these organisms, *Bacillus ixiae* G. S., and *Pseudomonas gladioli* G. S., in morphological or in cultural characters is like the organism, *Bacterium marginatum*, described in this paper.

Prillieux and Delacroix (*6 p. 670*) have published a very brief note concerning deeply corroded brown spots on the roots of "Glaieul". A short, very motile bacillus was found in the tissues. In bouillon cultures no color change took place in the medium. No name was given the organism and no further description has been found concerning this bacillus.

Rostrup (*8, p. 473; 9, p. 175*) refers to a disease of hyacinth and gladiolus which causes yellow stripes on the leaves in which bacteria have been found.

A yellow bacterium causing translucent spots on gladiolus leaves was described in 1924 by McCulloch (*3*). The appearance of the lesions and the characteristics of the causal organism are quite unlike *Bacterium marginatum*.

Mizusawa (*5*) reports a disease on crocus due to *Bacillus Croci* Mizusawa, and his photographs of colonies resemble *Bacterium marginatum* but the cultural characters and morphology of the bacteria are unlike *Bact. marginatum*.

Wallace (*11*) refers briefly to a disease of gladiolus, "a soft rot probably caused by bacteria." No description is given of the disease or the organism.

SUMMARY

The bacterial disease of gladiolus described in this paper has been under observation for several years in the

District of Columbia and near-by localities. This disease has been found on gladioli grown in Michigan, Ohio, Pennsylvania, Maryland, Virginia, Florida, California, and Indiana.

The leaf injuries vary from minute, reddish spots to large, brownish areas occurring most abundantly on the lower part of the leaves. The parenchyma is first destroyed and later the vessels become unable to supply sufficient moisture to the leaf which becomes brown and dry.

Infected corms are more or less disfigured by circular, shallow depressions. These are usually brown in color, horny or brittle in texture, and exude a gummy substance. The spots on the husks are brown to black, eventually disintegrating and exposing the body of the corm.

Isolations have been made from lesions on leaves, husks, corms, and from the exudate, and the pathogenicity of the bacteria from all these parts proved by inoculation experiments.

Living, virulent bacteria have been isolated from corm lesions 9 months after being harvested.

The organism causing these lesions is a medium-sized rod, having a capsule and one to several polar flagella. On beef media it forms a white, viscid growth.

This organism grows best at temperatures between 25° and 30° C. (77–86° F.). Moisture, heat, and succulent tissues favor the progress of the disease.

There is evidence that this organism remains alive in the soil in which diseased plants have grown. Crop rotation should be practiced.

Control experiments have not yet been extensive enought to indicate the best methods for prevention, but considerable success has resulted from treating the corms with either mercuric chloride (1–1,000) or formalin (1–80).

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NEW TERMITES AND HITHERTO UNKNOWN CASTES FROM THE CANAL ZONE, PANAMA [1]

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INTRODUCTION

The agricultural development of the Canal Zone and the clearing of the dense growth of tropical jungle for banana, pineapple, avocado, and cacao plantations will result in the killing of many termite colonies of species that require a moist habitat. The intense heat of the tropical sun will render the decaying logs, stumps, and branches on the ground and even the soil too dry and unsuitable for them. Large areas of the Zone already have been cleared of termites by the formation of Gatun Lake, which flooded the land, thereby drowning the termite colonies in the soil. Nevertheless, termites will always constitute a serious problem in Panama, and damage to the woodwork and contents of buildings as well as to living vegetation must be carefully guarded against.

The writer spent the month of February, 1924, in the Canal Zone and adjacent portions of the Republic of Panama, where several new and striking termites were collected by Messrs. J. Zetek, I. Molino, and himself. During his visit a new subgenus, Uniformitermes, of the genus Nasutitermes Banks was found, which contains two types of soldiers of similar form or shape. Species were found in two rare American genera, namely, Cylindrotermes Holmgren from Bolivia (species, *nordenskiöldi* Holmgren) and Rhynchotermes Holmgren (a subgenus of Armitermes Wasmann, from South America), hitherto monotypic (species, *perarmatus* Snyder); neither genus had been found previously in Panama.

The three species in the genus Cryptotermes Banks found in Panama in the latter part of the nineteenth century by Dudley and Beaumont, who gave no definite locality records, were rediscovered, and the hitherto unknown deälated adult of *Cryptotermes longicollis* Banks was found (fig. 2), which will be described after more material has been obtained. A study of *C. brevicollis* Banks (fig. 1) and *C. longicollis* Banks convinced the writer that they should be included in Holmgren's subgenus Lobitermes. *C. dudleyi*, on the other hand, is a Cryptotermes and suppresses *thompsonae* Snyder. Neither Banks's figures nor his description of *C. dudleyi* indicates that the soldier has the anterior margin of the pronotum serrate, the distinctive character of *C. thompsonae*, but the writer has since examined Banks's type.

Fig. 1.—*Kalotermes* (*Lobitermes*) *brevicollis:* Mandibles of soldier, showing marginal teeth. (From drawing made by camera lucida)

Interesting biological notes were obtained on the habits of Cylindrotermes and Rhynchotermes; Cylindrotermes (Pl. 1, B) lives under very similar conditions to species of Amitermes (*beaumonti*) and Leucotermes.

The termite fauna of Barro Colorado Island in Gatun Lake, Canal Zone, the site of the new tropical research station, was especially rich and interesting; 20 species, representing 15 genera or subgenera, occurring. At present only 36 species of termites, representing 22 genera or subgenera, have been found in Panama but it is believed that many more species occur. The outlying regions of the Republic of Panama have not been explored for termites and doubtless South American species will be found there. To the writer,

Fig. 2.—*Kalotermes* (*Lobitermes*) *longicollis:* Mandibles of soldier, showing marginal teeth. (From drawing made by camera lucida)

[1] Received for publication June 25, 1924—issued January, 1925.

Central America is an extremely interesting field for biological and taxonomic studies of termites.

Damage by termites to the woodwork of buildings in the Canal Zone is common and severe, while injury to living shade and fruit trees and other vegetation is serious, healthy living trees being attacked and killed. The Bureau of Entomology has therefore begun a series of experiments in the Canal Zone with wood preservative treatments to prevent termite damage to buildings, as well as tests of insecticides to kill Coptotermes infesting trees. J. Zetek, of this bureau, stationed at Ancon, Canal Zone, and the writer are cooperating in this work, some of which, through the courtesy of the Institute for Research in Tropical America, will be conducted at the site of their station on Barro Colorado Island.

The seven new species are *Kalotermes panamae*, *K.* (*Glyptotermes*) *angustus*, *K.* (*G.*) *barbouri*, and *K.* (*G.*) *emarginicollis*, in the family Kalotermitidae, and *Armitermes* (*Armitermes*) *chagresi*, *A.* (*Rhynchotermes*) *perarmatus*, and *Nasutitermes* (*Uniformitermes*) *barro-coloradensis*, in the family Termitidae.

A list of the known termites of the Canal Zone and adjacent Panama is appended. The present paper will be followed shortly by a longer biological paper discussing these termites, their distribution, economic importance, and control.

HABITS AND HABITATS

Unlike ants, termites [2] are not dominant insects. They seldom come above ground to forage in the direct sunlight, and species which do forage above ground are specially modified. The deeply pigmented, sexual adults possess eyes and are phototropic at the time of the annual colonizing flight, known as the "swarm"; but after the flight, which is generally of short duration, the deälated adults rapidly become thigmotropic and seek shelter in or under decaying wood on the ground or in crevices in dead trees. Most termites are blind and live hidden within wood, underground, or in carton tree nests or mound nests on the ground.

Colonies occur under a great variety of habitats—in the sands near sea coasts, in forests, swamps, plains, arid deserts, fields, orchards, and cities. By the clearing of forest land in temperate regions, subterranean termites are often driven to attack the crops and buildings of man. In the moist tropical jungle, subterranean termites inhabiting wood on the ground are often killed by the intense heat of the sun in clearings for crops.

As a rule termites of subterranean habit must come above or near the surface of the ground to procure their food, which consists of wood, vegetation, or roots. To solve this difficulty, these soft-bodied, blind insects take the ground and moisture out with them. Even when working in the tops of lofty trees they are thus "below ground."

For protection and moisture they construct earthlike carton shelter tubes of excreted wood and soil cemented together. Occasionally these run along the ground, but more often mount in extensive ramifications to the tops of the trees, meandering along every branch and twig, and here and there debouching into large covered chambers which occupy half the girth of the trunk. Most trees in some regions are thus fantastically plastered over with tubes, galleries, chambers of earth, and carton nests.

Hence it will be seen that wood, no matter how dry, may be attacked by subterranean termites if there is access to the wood from the soil whence they can obtain moisture.

GEOGRAPHICAL DISTRIBUTION

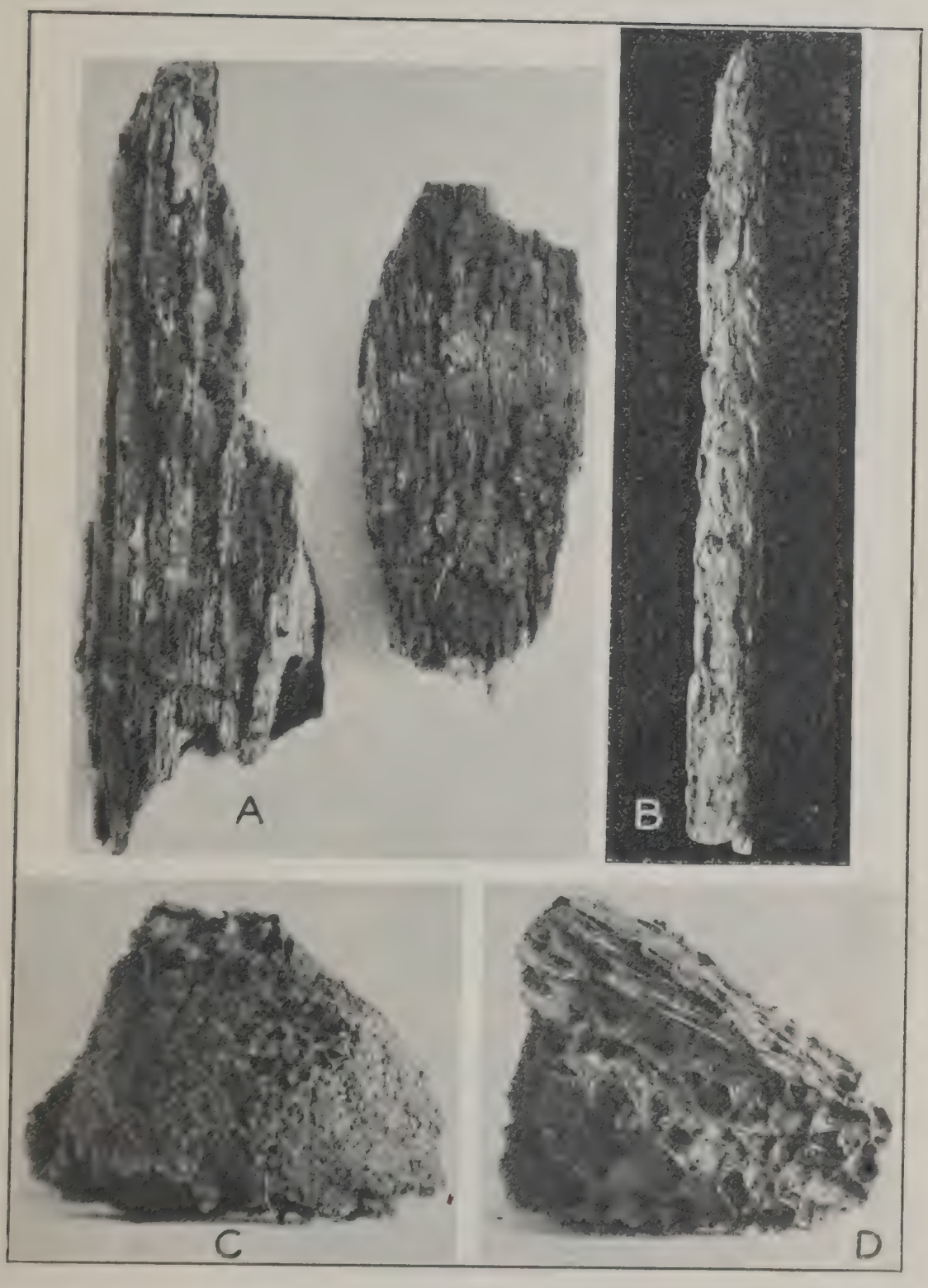
Termites, although widespread, are not to be found in arctic regions or above timber line on mountains. They occur in the temperate regions of the world and in the tropics and subtropics, where termites reach their maximum development. They have not the wide range of distribution of ants, nor are there as many species of termites as there are of ants.

The more permanent nests and more stable colonies occur in the tropics. Colonies in temperate regions have but poorly defined nests and are more mobile; in the tropics specialized nest structures are the rule.

SOCIAL LIFE

Unlike colonies of ants, termite colonies are societies of sexual and sterile castes of both sexes. The male continues to cohabit with the female and copulation is repeated at intervals throughout life. Although soft-bodied and usually blind and sensitive to light, termites are the longest-lived of all insects. Reproductive adults may live

[2] Termites, or "white ants," are termed "comixen" or "comejen" in Cuba and Spanish Central America and South America, but are termed "cupim" in Brazil.



New Termites from the Cana lZone

Plate 1

A.—*Armitermes* (*Rhynchotermes*) *perarmatus:* Work in decaying log on ground in jungle.
B.—*Cylindrotermes nordenskiöldi:* Work in branch on ground in jungle.
C, D.—*Mirotermes panamaensis:* Exterior and interior views of nest within dead coconut palm tree on ground where reproductive forms were found, Largo Remo Island, Canal Zone.
Photographs by Zetek and Molino.

for 25 years or more. There is a well-defined division of labor among workers, soldiers, and reproductive forms; all castes are polymorphic.

ECONOMIC IMPORTANCE

Termites are very destructive to the crops, habitations, and other works of man and cause millions of dollars worth of damage. The greatest usefulness of termites is in reducing wood to humus and turning over and aerating the soil. They hasten the decomposition of organic matter such as dead trees, decaying logs, and stumps, thus enriching the soil. In the tropics of Africa and India termites plow and harrow the soil in their burrowings underground, and vegetation taken as food by termites passes through their bodies to enrich the soil. By their subterranean excavations, also, the soil is kept in the constant circulation so conducive to proper productiveness. Without the angleworms or earthworms, ants, and termites, the soil might become barren and incapable of supporting human life.

Although the motivations in the termite colony life are hunger, sex, and fear, there are evidences that the workers regulate the life of the colony and are the "spirit of the colony," as Maeterlinck has so aptly termed the guiding force of colony life in the bee. Some of the actions of the workers, however, can not be attributed to these impulses alone, as, for example, the seasonal slaughter of certain types of reproductive forms. There is a definite selective process in these killings which apparently works out for the welfare of colony life.

FOOD

Cellulose is the chief food of termites and this is obtained not only from living vegetation (living trees, roots, etc.) but also from dead vegetation as dead trees, plants, paper, etc. Recent investigations by Cleveland have shown that most termites have protozoa in the intestinal tract and these act as enzymes in the digestion of this cellulose. Most termites of the family Termitidae, however, do not have protozoa in the intestinal tract although their food is in general similar to that of other termites.

INSECT ENEMIES

Ants are the greatest insect enemies of termites and yet certain species of ants are pacific toward termites and live in their nests, although in separate galleries. No internal insect parasites of termites have been found and possibly this is due to the fact that termites have no resting stage (as immobile larvæ or pupæ) and the young or nymphs are constantly active, except for short molting periods or quiescent stages.

ANIMAL PREDATORS

A great variety of odd and specially adapted animals prey on termites. The most interesting of these are the anteaters, and America contains several species of these peculiar mammals, the largest of which is *Myrmecophaga tridactyla* Linnaeus. Dr. W. M. Mann found another species, *Cyclopes didactylus* Linnaeus, a small, golden-yellow arboreal animal, feeding on termites in Bolivia. A larger species, *Tamandua tetradactyla* Linnaeus (Pl. 2, A, B), also arboreal, preys on termites and often rare species of termites, ants, and inquilines are found in the stomachs of these anteaters if they are killed before the stomach contents are digested.

DESCRIPTIONS OF NEW SPECIES OF TERMITES AND HITHERTO UNKNOWN CASTES OF KNOWN SPECIES

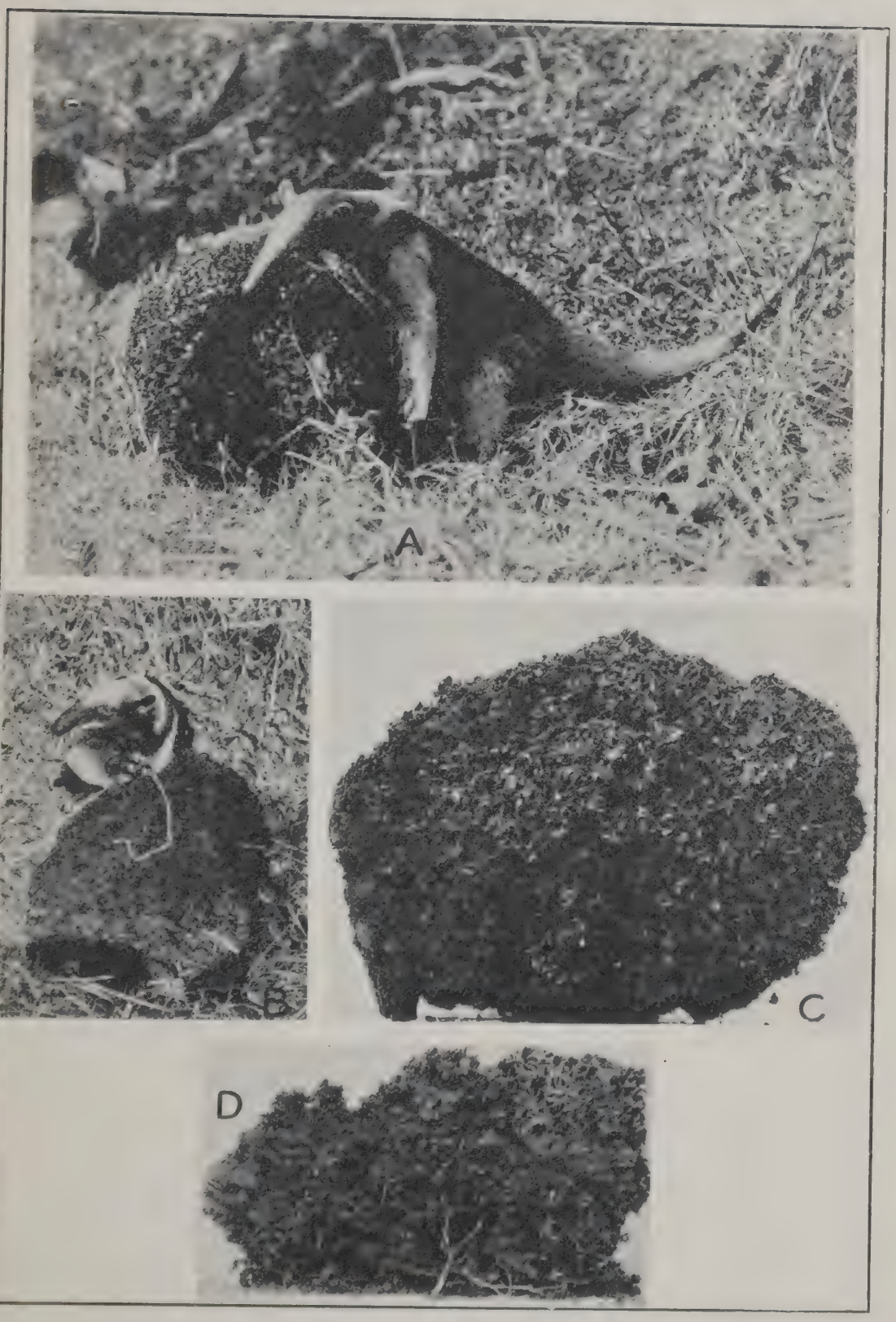
Kalotermes panamae, new species.

SOLDIER.—Head yellowish-brown, darker anteriorly, over twice as long as broad, broadest posteriorly, slightly narrower anteriorly, sides parallel (slightly concave in middle), a chitinized projection or knob between antennal socket and postclypeus, head with very dense fairly long hairs; eye spot hyaline, elongate, suboval, parallel to front of head, epicranial suture on slope of front of head, where there is a slight depression; labrum rounded (broadly) at apex. Gula one-half as wide at middle as at front.

Mandibles blackish, base reddish brown, broad at base, incurved at apex; left mandible with two prominent pointed teeth near apex and three smaller teeth nearer base; right mandible with two prominent teeth near base (fig. 3).

Antennae yellowish, third segment castaneous, of 14 segments, with long hairs; third segment subclavate, approximately as long as fourth and fifth segments together; last segment slender and subelliptical.

Pronotum yellowish, margin darker, as broad as head, broader than long (variable in size), broadly emarginate



New Termites from the Canal Zone

Plate 2

A, B.—*Tamandua tetradactyla*, an arboreal American anteater, in whose stomach were found rare termites, feeding on a carton termite nest.

C, D.—*Armitermes* (*Armitermes*) *chagresi:* View of exterior and section of termitarium 20 inches wide. of soft earth on ground near decayed log in jungle, Barro Colorado Island, Canal Zone.

Photographs by Zetek and Molino.

anteriorly, not as deeply as in *K. marginipennis* Latreille, and slightly emarginate posteriorly, anterior margin *not* dentate (fig. 4), sides rounded, with dense, fairly long hairs.

Legs yellowish, tibiae slightly brownish, femora swollen, pubescent.

Abdomen dirty whitish gray, pubescent, with row of long hairs on tergites near base; cerci present but not prominent.

MEASUREMENTS.—Length of entire soldier: 9.50–11.00 mm. Length of head with mandibles: 4.60–4.80 mm. Length of head to anterior margin: 2.90–3.50 mm. Length of left mandible: 1.60–1.70 mm. Length of pronotum: 1.10–1.30 mm. Length of hind tibia: 1.30–1.40 mm. Width of head (where widest): 1.80–2.00 mm. Width of pronotum: 1.80–2.20 mm.

TYPE LOCALITY.—Rio Chinilla, Canal Zone, Panama.

FIG. 3.—*Kalotermes panamae:* Mandibles of soldier, showing marginal teeth. (From drawing made by camera lucida)

FIG. 4.—*Kalotermes panamae:* Dorsal view of pronotum of soldier, the anterior margin *not* dentate. (From drawing made by camera lucida)

Described from six soldiers, collected with nymphs in a decaying log in jungle, at the type locality, by I. Molino and T. E. Snyder on February 22, 1924.

TYPE, SOLDIER.—Cat. No. 27266, U. S. National Museum.

Neotermes holmgreni Banks.

SOLDIER.—Head light castaneous brown, paler posteriorly, with reddish tinge anteriorly, elongate, sides slightly concave, broader anteriorly, frontal slope with lobed epicranial suture, eye spot hyaline, elongate, parallel to frontal slope, head with scattered long hairs. Gula slender in middle.

Mandibles black, base reddish, broad at base, more slender toward apex; incurved at apex; right mandible with two sharp, pointed teeth near base, slightly crenulate toward apex; left mandible with three "molar" (double) teeth with first teeth more prominent.

Antennae light brown, paler anteriorly, not as long as mandibles, of 13 segments, with long hairs; third segment slightly modified, subclavate, slightly longer and broader than second and much longer and broader than fourth; fifth longer than fourth, segments becoming longer toward apex; last segment slender, shorter and subelliptical.

Pronotum gray-white with pale yellowish tinge, darker (brownish) on margins, anterior margin broadly, roundly but shallowly emarginate (concave), posterior margin convex, sides rounded, anterior corners rounded and somewhat raised, mesonota and metanota with very short wing pads, with scattered fairly long hairs.

Legs white with yellowish tinge, short, femora slightly swollen, pubescent, pulvillus absent.

Abdomen dirty white with yellowish tinge, with scattered shorter hairs and a row of long hairs near base of each tergite, cerci short.

MEASUREMENTS.—Length of entire soldier: 9.5–11.5 mm. Length of head with mandibles: 5.1 mm. Length of head to anterior margin: 3.3 mm. Length of left mandible: 1.8 mm. Length of pronotum: 1.2 mm. Length of hind tibia: 1.2 mm. Width of head: 2.1–2.2 mm. Width of pronotum: 2.1–2.2 mm.

Described from a series of soldiers collected with nymphs at the type locality (Taboga Island, Republic of Panama) by the writer on February 17, 1924, in a decaying branch on the ground. These specimens are in the collection of the United States National Museum. No winged adults were collected with these soldiers. The winged adult was described by Nathan Banks in 1918, and these are the first soldiers collected, this caste being hitherto unknown. Specimens of soldiers from Ancon Hill, Canal Zone, show variations in mandibular teeth, pronotum, etc.

Kalotermes (Glyptotermes) angustus, new species.

WINGED ADULT.—Head shining, dark castaneous brown, lighter below the eyes, longer than broad, sides parallel, with scattered long hairs; eyes black, not round, large, slightly projecting, separated from lateral margin of head by a distance less than half an eye diameter; ocelli hyaline, small suboval, close to and at an oblique angle to eye.

Antennae light brown, of 12 segments, with long hairs; segments becoming longer and broader toward apex; third segment subclavate, longer than fourth but shorter than second; last segment longest, apex appearing to be somewhat obliquely truncated.

Pronotum same color as head, anterior margin broadly, shallowly, roundly emarginate, sides sloping roundly to posterior margin, where slightly emarginate, with few scattered long hairs.

Wings with costal veins brownish, no branches between costal veins, membrane yellowish, coarsely punctate; forewing with median close to and parallel to subcostal vein, cubitus slightly nearer to median than to lower margin (a little above middle of wing), branching to apex of wing, with subbranches to lower margin.

Legs light yellow-brown, pulvillus present, pubescent.

Abdomen with tergites same color as head, a row of long hairs at base of each tergite, cerci present, but not prominent.

MEASUREMENTS.—Length of entire winged adult: 6.00 mm. Length of entire deälated adult: 3.50 mm. Length of head: 1.00 mm. Length of pronotum: 0.50 mm. Length of anterior wing: 4.30 mm. Length of hind tibia: 0.70 mm. Diameter of eye (long diameter): 0.225 mm. Width of head: 0.75 mm. Width of pronotum: 0.70 mm. Width of anterior wing: 1.20 mm.

SOLDIER.—Head light castaneous brown, paler posteriorly, blackish on front, which is nearly vertical, longer than broad, broadest posteriorly, sides approximately parallel, slightly concave at middle, constricted at top of head anteriorly, deeply bilobed medially (narrow V-shaped cut), rims of lobes roughened, tuberculate, surface of head smooth, with scattered long hairs. Eye spot hyaline, large, markedly projecting, suboval, and parallel to front margin of head.

Mandibles reddish brown to blackish at apex, short, stout, slender and curved at apex; left mandible with three marginal teeth, the two near apex approximately parallel to margin of mandible, molar area bare; right mandible with sharp-pointed projecting tooth at about middle and molar area bare.

Antennae light yellow-brown, of 11 segments, segments wedge-shaped, becoming longer and broader toward apex, with long hairs; third segment longest, next shorter than second and little shorter than fourth; last segment long and slender, subelliptical and pointed at apex.

Pronotum yellow-brown, darker on margins, narrow, anterior margin broadly, roundly, fairly deeply emarginate, slightly roughened, sides high, sides roundly sloping to posterior margin, where roundly emarginate, with few scattered long hairs (fig. 5, *a*).

Legs whitish with tinge of yellow, femora swollen, pubescent. Abdomen dirty gray-white, with tinge of yellow, tergites with a row of long hairs near bases, cerci not prominent.

MEASUREMENTS.—Length of entire soldier: 4.40–4.80 mm. Length of head with mandibles: 1.80 mm. Length of head to anterior margin: 1.30 mm. Length of left mandible: 0.65 mm. Length of pronotum: 0.45–0.50 mm. Length of hind tibia: 0.70 mm. Width of head posteriorly: 0.95 mm. Width of head anteriorly: 0.90 mm. Width of pronotum: 0.95 mm.

TYPE LOCALITY.—Rio Chinilla, Canal Zone, Panama.

Described from a series of winged adults and soldiers collected at the type locality in a decaying log on February 22, 1924, by T. E. Snyder and I. Molino.

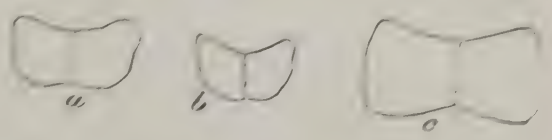


FIG. 5.—Comparative study of dorsal views of pronota of soldiers of (*a*) *Kalotermes (Glyptotermes) angustus*, (*b*) *K. (G.) barbouri*, and (*c*) *K. (G.) emarginicollis*. (All from camera lucida drawings made to same scale)

TYPE, SOLDIER.—Cat. No. 27267, U. S. National Museum; morphotype, winged adult.

Kalotermes (Glyptotermes) barbouri, new species.

DEÄLATED MALE ADULT.—Head shining, dark castaneous brown, lighter below eyes, slightly longer than broad, sides parallel, with fairly dense scattered long hairs; eyes black, large, slightly projecting, suboval, separated from lateral margin of head by a distance less than an eye diameter; ocelli hyaline, small, suboval parallel to and close to eye.

Antennae broken, yellow brown, with long hairs; third segment subclavate, longer than second or fourth segments; fourth shorter than second segment; segments becoming longer and broader toward apex.

Pronotum same color as head, anterior margin shallowly roundly emarginate, sides roundly sloping to posterior margin, which is nearly straight, with scattered long hairs.

Wings with costal area golden brown, membrane whitish with yellowish tinge, coarsely punctate; hind wing

with median vein close to and parallel with the subcostal vein, cubitus in about center of wing, branching to apex of wing, near base branching into a long vein parallel to cubitus, and with subbranches to lower border of wing.

Legs yellow, tibiae yellow-brown, pubescent.

Abdomen with tergites same color as head, a row of long hairs near base of each tergite; cerci present but not prominent.

MEASUREMENT.—Length of entire deälated adult: 3.70 mm. Length of head: 0.90 mm. Length of pronotum: 0.50 mm. Length of hind tibia: 0.70 mm. Length of hind wing: 4.20 mm. Diameter of eye (long diameter): 0.225 mm. Width of head (at eyes): 0.80 mm. Width of pronotum: 0.70 mm. Width of hind wing: 1.15 mm.

SOLDIER.—Head pale yellowish brown, darker anteriorly (castaneous or reddish brown), blackish on front, which is nearly vertical, longer than broad, broadest posteriorly, sides approximately parallel but slightly narrowed anteriorly, especially constricted on top of head, deeply bilobed medially, with rims of lobes roughened or tubercular, surface of head smooth, with few scattered long hairs. Eye spot hyaline, large, projecting, elongate, suboval, parallel to front of head.

Mandibles reddish brown to blackish, short, stout, incurved at apex; left mandible with three marginal molar teeth, tooth near apex with more pointed, projecting base; right mandible with one molar tooth near base, with fairly sharp, projecting base.

Antennae light yellowish brown, of 10 to 11 segments, segments becoming longer and broader toward apex (wedge-shaped), with long hairs; if 10 segments, third segment longer than fourth but shorter than second; if 11 segments (third dividing), third segment ringlike, shorter than second or fourth; last segment narrow, elongate and subelliptical, pointed at apex.

Pronotum light yellow-brown, darker on margins, anterior margin deeply roundly emarginate, corners high, sides roundly sloping to posterior margin, where nearly straight (slightly emarginate), with few scattered long hairs (fig. 5, *b*).

Legs whitish with tinge of yellow, femora slightly swollen, pubescent.

Abdomen dirty white with tinge of yellow, tergites with a row of long hairs near base, cerci present but not prominent.

MEASUREMENTS.—Length of entire soldier: 4.10–4.20 mm. Length of head with mandibles: 1.60–1.75 mm. Length of head to anterior margin: 1.20–1.30 mm. Length of left mandible: 0.70 mm. Length of pronotum: 0.50–0.55 mm. Length of hind tibia: 0.55 mm. Width of head (at widest part): 0.85 mm. Width of pronotum: 0.70–0.75 mm.

TYPE LOCALITY.—Barro Colorado Island, Canal Zone, Panama.

Described from two soldiers and one deälated adult collected with nymphs at the type locality by I. Molino and T. E. Snyder on February 21, 1924, in a decaying branch lying on the ground in moist jungle. This species was named in honor of Dr. Thos. Barbour, of the Museum of Comparative Zoology, Cambridge, Mass.

TYPE, SOLDIER.—Cat. No. 27269, U. S. National Museum; morphotype, deälated adult.

Kalotermes (Glyptotermes) emargini collis, new species.

SOLDIER.—Head light castaneous brown, yellow-brown on sides and posteriorly, reddish brown to blackish anteriorly, front blackish, nearly vertical, head longer than broad, broadest posteriorly, sides approximately parallel, constricted on top of head anteriorly, rim elevated, front deeply bilobed medially with a wide U-shaped cut, lobes not roughened, surface of head smooth, with few scattered long hairs. Eye spot hyaline, large, somewhat projecting, suboval, parallel to front of head.

Mandibles blackish, base reddish brown, short, stout, incurved at apex; left mandible with three pointed marginal teeth; right mandible with two prominent pointed marginal teeth.

Antennae white with tinge of yellow, of twelve segments, segments wedge-shaped, becoming longer and broader toward apex, with long hairs; third segment ringlike, shorter than second or fourth segments; last segment slender, subelliptical, pointed at apex.

Pronotum light yellow-brown, darker on margins, margins deeply roundly emarginate, corners high anteriorly and roundly sloping posteriorly, with scattered long hairs (fig. 5, *c*).

Legs yellowish, femora slightly darker dorsally, swollen, three prominent reddish brown spines on tibia of front legs at apex, with hairs; prosternal processes dark (castaneous).

Abdomen dirty gray-white with yellowish tinge; tergites with dense short hairs and a row of long hairs at base of each tergite; cerci present but not prominent.

MEASUREMENTS.—Length of entire soldier: 5.50 mm. Length of head with mandibles: 2.50–2.60 mm. Length of head to anterior margin: 2.10–2.20 mm. Length of left mandible: 0.90. Length of pronotum: 0.80–0.90 mm. Length of hind tibia: 0.85 mm. Width of head: 1.40–1.55 mm. Width of pronotum: 1.40 mm.

TYPE LOCALITY.—Rio Chinilla, Canal Zone, Panama.

The specific name is derived from the emarginate pronotum of the soldier; this is a larger species than *K.* (*G.*) *pubescens* Snyder of Porto Rico and the soldier has a markedly emarginate pronotum.

Described from a series of soldiers collected with nymphs in a decaying log on the ground at the type locality by T. E. Snyder and I. Molino, on February 22, 1924.

TYPE, SOLDIER.—Cat. No. 27268, U. S. National Museum.

Cornitermes acignathus Silvestri.

DEÄLATED MALE ADULT.—Head castaneous, round, with a hyaline, suboval, raised fontanelle, in a depression (larger than an ocellus); head with few scattered long hairs. Eye black, large projecting, not quite round, very near lateral margin of head. Ocellus hyaline, large, suboval, upper rim projecting, separated from eye by a distance about one-half the small diameter of an ocellus.

Antennae yellow-brown, 15-segmented, with long hairs; third segment subclavate, longer than second or fourth segments; last segment slender, subelliptical.

Pronotum same color as head, anterior margin straight, posterior margin emarginate, narrowing posteriorly, hyaline "spread-wing"-shaped markings, anteriorly centering on median line, an elliptical marking at each anterior corner, and two elliptical markings near posterior margin separating from a common base at the median line to form an acute angle, diverging from the median line toward the sides; with dense long hairs.

Legs yellow-brown, tibiae darker, elongate, slender, pubescent.

Abdomen with tergites castaneous, two spiracular, hyaline markings about one-third way toward median line, one at each side in center of tergite (more prominent than in *C. striatus* Hagen), with long hairs near base of tergites, cerci not very prominent.

MEASUREMENTS.—Length of entire deälated male adult: 8.50 mm. Length of head: 2.15 mm. Length of pronotum: 1.05 mm. Diameter of eye (long diameter): 0.70 mm. Diameter of hind tibia: 2.60 mm. Width of head (at eyes): 2.15 mm. Width of pronotum: 2.00 mm. Length of mature queen: 18.50 mm. Width of abdomen of mature queen: 6.50 mm.

The deälated adult of *C. acignathus* is lighter colored, and larger than that of *C. striatus* Hagen, with larger fontanelle and eyes, ocellus more distant from eye, and markings on pronotum different. The species *C. acignathus* has been hitherto known only from the soldier caste from which it was described by Silvestri in 1903, although Nathan Banks states that the adult is similar to *C. striatus*, with a slender fontanelle.

Described from mature, deälated males and females (kings and queens) of the first form collected at Rio Tapia, Republic of Panama, on February 7, 1924, by the writer with soldiers and workers in decayed logs on the ground in the jungle. Other specimens were collected by the writer at Rio Chinilla, Canal Zone, Panama, on February 22, 1924. These specimens are all deposited in the collection of the United States National Museum.

Armitermes (Armitermes) chagresi, new species.

SOLDIER.—Head light yellow, nearly as broad as long, head and nasus not convex in profile, slightly concave at base of nasus, sides convex, broadest near posterior margin, narrowed anteriorly; with scattered long hairs. Nasus light yellow-brown, fairly robust, broad at base, narrowing anteriorly, dense short hairs at apex, not as long as head (in *A. armigera* Motschulsky nasus is longer than head). Mandibles slender and strongly curved, with slightly outward pointing marginal tooth nearer base than apex (fig. 6).

Antennae light yellow-brown, of 14 segments, pubescent, extending far beyond tip of nasus; third segment subclavate, longer than second or fourth segments; segments becoming longer and broader toward apex; last segment short, slender, subelliptical. Gula not much narrowed in middle.

Pronotum white, tinged with yellow, saddle-shaped, emarginate anteriorly, sides sloping roundly to posterior margin, which is rounded, with scattered long hairs.

Legs tinged with yellow, fairly elongate, slender, pubescent.

Abdomen tinged with yellow, densely pubescent; cerci prominent.

MEASUREMENTS.—Length of entire soldier: 5.50–5.65 mm. Length of head with nasus: 2.30–2.40 mm. Length of head with mandibles: 2.40–2.50 mm. Length of head to anterior margin: 1.50 mm. Length of nasus: 0.85 mm. Length of left mandible: 1.10 mm. Length of pronotum: 0.35 mm. Length of hind tibia: 1.30 mm. Width of head (at widest point posteriorly): 1.35–1.40 mm. Width of head at anterior margin: 0.95 mm. Width of pronotum: 0.75 mm.

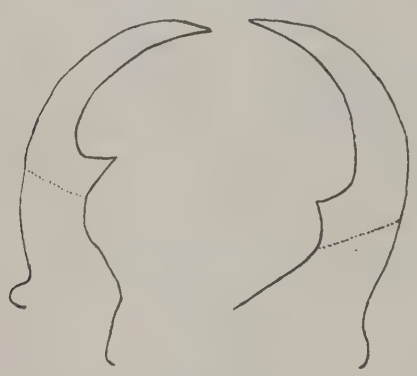


FIG. 6.—*Armitermes* (*Armitermes*) *chagresi:* Mandibles of soldier, showing marginal teeth. (From drawing made by camera lucida)

Specimens of *A.* (*A.*) *chagresi* Snyder were first found at Barro Colorado Island, Canal Zone, on February 21, 1924, by T. E. Snyder in a carton nest on the ground in the jungle (Pl. 2, C, D). Formerly *A.* (*A.*) *armigera* Motschulsky was considered to be the only species in Panama; it was described from Obispo and I have seen the type at the Museum of Comparative Zoology, Cambridge, Mass.; *chagresi* is close to *neoteinicus* Holmgren and *percutiens* Emerson; the antennae are also different from those in *armigera.* Specimens of *chagresi* were also collected at Rio Chinilla, Canal Zone, on February 22, in a carton nest and in decaying logs; in these specimens the nasus is slightly longer and the head and nasus tend toward the convex in profile. In the genus Armitermes there appear to be either a large number of closely related species or much variation within the species.

TYPE LOCALITY.—Barro Colorado Island, Canal Zone, Panama.

Described from a series of soldiers collected with workers at the type locality by T. E. Snyder on February 21, 1924.

TYPE, SOLDIER.—Cat. No. 27357, U. S. National Museum.

GENUS ARMITERMES WASMANN

SUBGENUS RHYNCHOTERMES HOLMGREN

In 1912, Holmgren established the subgenus Rhynchotermes for Silvestri's remarkable species *nasutissimus* from South America; this subgeneric name, derived from the Greek ῥύγχος (beak), is very appropriate and descriptive. This subgenus is monotypic. A much more striking and longer-beaked species has recently been found in Panama; the marginal teeth to the mandibles of the soldier are elongate and sharp-pointed and the anterior process on the coxa of the forelegs is more bent and hook shaped. It is a thoroughly armed species and runs about audaciously with its nasus or beak elevated at an angle of 45°, reminding one of an antiaircraft gun.

Armitermes (Rhynchotermes) perarmatus, new species.

SOLDIER (fig. 7). Head light castaneous brown, middle of beak and mandibles reddish brown, head short, pear-shaped, nasus very elongate, curved downward and becoming gradually attenuated toward apex; one row of hort hairs on head anteriorly. Mandibles very short, bent inward or hook-shaped, inner margin near tips not crenulate, near center of each mandible a long, outward-curved, sharp-pointed marginal tooth.

Antennae yellow-brown, elongate, slender, of 14 segments, with long hairs; third segment subclavate, as long as first, longer than second or fourth; fourth segment longer than second; fifth segment longer than fourth; segments gradually becoming shorter but broader toward apex; last segment subelliptical.

Pronotum light yellow-brown, darker on margins, saddle-shaped, slightly broader than long, high and roundly narrow at anterior margin, where emarginate, broadly rounded posteriorly.

Legs with tinge of yellow, elongate, with scattered long, but sparse, hairs, coxa of foreleg with an elongate point or sharp process, sometimes hook shaped and curving; two spines at apex of tibia.

Abdomen dirty gray-white, with tinge of yellow, with row of long hairs at base of each tergite.

MEASUREMENTS.—Length of entire soldier: 4.50–4.60 mm. Length of head with nasus: 2.50–2.60 mm. Length of head with mandibles: 1.20 mm. Length of head to anterior margin: 1.00 mm. Length of nasus: 1.60 mm. Length of left mandible: 1.05 mm. Length of pronotum: 0.40 mm. Length of hind tibia: 1.50 mm. Width of head (at widest portion): 0.90–0.95 mm. Width of pronotum: 0.55 mm.

The soldier of *A.* (*R.*) *perarmatus* is darker colored, larger than *nasutissimus* Silvestri, and has a longer, more aquiline head and nasus, longer mandibles, and long, pointed marginal teeth. The antennae have longer segments, and the process on the coxae is longer and more pointed.

WORKER.—Darker colored and larger than that of *nasutissimus;* head yellow-brown, with prominent fontanelle; antennae with 14 segments; postclypeus bulging.

MEASUREMENTS.—Length of entire worker: 4.1 mm. Length of head: 1.50 mm. Length of pronotum: 0.45 mm. Length of hind tibia: 1.50 mm. Width of head: 1.30 mm. Width of pronotum: 0.70 mm.

The winged adult is unknown.

TYPE LOCALITY.—Rio Chinilla, Canal Zone, Panama.

Described from a large series of soldiers and workers collected in a decayed log on February 22, 1924, at the type locality by I. Molino, J. Zetek, and T. E. Snyder; two colonies collected.

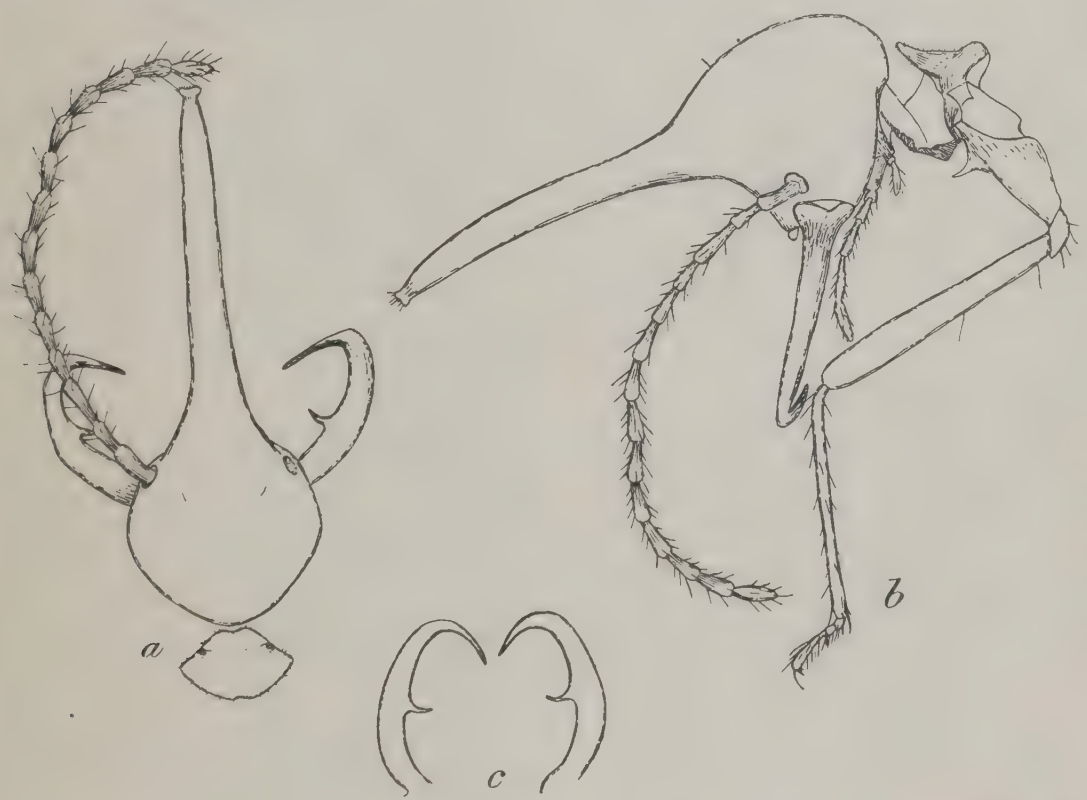


FIG. 7.—*Armitermes* (*Rhynchotermes*) *perarmatus*, soldier: *a*, Dorsal view of head and prothorax, showing long nasus and pointed marginal teeth of mandibles; *b*, lateral view of same, showing elongate, curved nasus, mandible, coxal spine of long foreleg, and antenna; *c*, dorsal view of mandibles

TYPE, SOLDIER.—Cat. No. 27240, U. S. National Museum.

BIOLOGICAL NOTES.—This termite lives in bark-covered decaying logs in the moist, dense jungle; it was not found in earthlike carton nests. Sometimes several other species of termites inhabit the same log, as *Coptotermes niger* Snyder and *Rhinotermes longidens* Snyder. A species of Peripatus was also found in a log inhabited by *A.* (*R.*) *perarmatus* (Pl. 1, A).

Dr. W. M. Mann found specimens of this termite at Sangrelaya, Honduras, in May, 1924, under bark in a decayed log in the jungle.

A single winged male adult of a species of Rhynchotermes was collected

flying by August Busck at Paraiso, Canal Zone, Panama, on April 24, 1911, and is now in the collection of the United States National Museum. This may be *Armitermes* (*Rhynchotermes*) *perarmatus*, as *perarmatus* is the only species so far collected in Panama. *A.* (*R.*) *nasutissimus* Silvestri of South America appears to be different. It is not *A.* (*Armitermes*) *armigera* Motschulsky, as it was tentatively and doubtfully determined by Nathan Banks in 1918.

WINGED MALE ADULT.—Head castaneous brown, longer than broad, rounded posteriorly, with a few scattered long hairs. Fontanelle hyaline. tinged with yellowish, a raised round spot in a depression, smaller than an ocellus. Eye black, large, projecting, not round; ocelli hyaline, elongate, projecting, close to eyes, on rim of frontal slope. Postclypeus lighter colored than head, bilobed, projecting.

Antennae yellow-brown (broken), with long hairs; third segment longer than second or fourth; fourth slightly longer than second; fifth and fourth subequal.

Pronotum yellow-brown, anterior margin slightly elevated, nearly straight, sides roundly sloping to posterior margin, which is broadly rounded and emarginate.

Wings smoky yellow, costal veins golden yellow, margins ciliate, wing surface *hairy*. Forewing with median vein nearer to cubitus than to subcosta, branching to apex of wing, cubitus nearly in center of wing, not reaching apex, with 11 branches or sub-branches to margin of wing.

Legs yellow-brown, slender, elongate, pubescent.

Abdomen light yellow-brown, with a row of dense long hairs at base of each tergite; cerci fairly prominent.

MEASUREMENTS.[3]—Length of entire winged adult: 15.00 mm. Length of entire deälated adult: 7.00 mm. Length of head: 1.90 mm. Length of pronotum: 0.80 mm. Length of forewing: 13.00 mm. Length of hind tibia: 2.00 mm. Diameter of eye (long diameter): 0.45 mm. Width of head (at eyes): 1.60 mm. Width of pronotum: 1.40 mm. Width of forewing: 4.80 mm.

The wing is hairy, whereas that of *A.* (*R.*) *nasutissimus* Silvestri is *not* hairy. *A.* (*R.*) *perarmatus* also has longer hind tibiae than *nasutissimus*. Comparison was made with a winged male adult of *nasutissimus* determined by F. Silvestri and donated by him to the United States National Museum, No. 47.

Nasutitermes (Obtusitermes) biformis Snyder.

DEÄLATED ADULT.—Head dark brown, with a reddish tinge, suboval, fairly flat, a narrow slope toward anterior margin, with dense, long hairs. Fontanelle a hyaline, narrow slit in a depression on vertex at about middle of eyes. Eyes black, nearly round, prominent, very near lateral margin of head; ocelli hyaline, suboval, fairly large, inset, at an oblique angle to eyes and separated from eyes by a distance less than the small diameter of an ocellus. Left mandible reddish-brown, first and second teeth approximately subequal. Postclypeus yellow-brown, not prominently arched, length less than half the width.

Antennae yellow-brown, of thirteen (+?) segments, with long hairs; third segment very short, ringlike; fourth shorter than second; segments becoming longer and broader toward apex, but all more or less wedge-shaped and fairly short.

Pronotum of same color as head, very slightly saddle-shaped, almost subcordate, anterior margin almost straight, posterior margin emarginate, sides rounded and sloping roundly to posterior margin, with dense long hairs.

Wing scale nearly as long as pronotum.

Legs light yellow-brown, fairly slender, pubescent.

Abdomen with tergites slightly lighter colored than head, with dense long hairs; cerci fairly prominent.

MEASUREMENTS.—Length of entire deälated adult: 5.50 mm. Length of head: 1.00 mm. Length of pronotum: 0.50. Length of hind tibia: 0.80 mm. Diameter of eye (long diameter): 0.25 mm. Width of head (at eyes): 0.95 mm. Width of pronotum: 0.70 mm.

Described from two deälated colonizing adults (male and female) collected in a small cavity in a decayed branch on the ground at Barro Colorado Island, Canal Zone, by J. Zetek and I. Molino on August 22, 1923; they are probably a young royal couple. *O. biformis* was described in 1924 from the soldier caste alone, the type locality being Quipo, Republic of Panama; these specimens were not found with soldiers, but soldiers and workers were in the branch,

[3] They were made from a dry, pinned specimen.

and are the only deälated adults of this species that have been collected; they are deposited in the collection of the United States National Museum. The winged adult is unknown.

GENUS NASUTITERMES BANKS

SUBGENUS UNIFORMITERMES, NEW SUBGENUS

In Panama another species of Nasutitermes has been found that does not agree with the characters of any known subgenus; this species, with others from Bolivia, intermediate between Diversitermes Holmgren and Velocitermes Holmgren, throws considerable doubt on the validity of subgenera in Nasutitermes. For the present, however, I prefer to adhere to the subgenera established by Holmgren and others; possibly this species is a Diversitermes.

Uniformitermes, new subgenus.

SOLDIER.—Two types of soldiers, but of somewhat the same general form, in each form head markedly constricted behind antennae. Nasus elongate, slender, cylindrical. Mandibles with points, sharp but not very elongate. The shape of the major soldier is somewhat similar to that of the minor soldier of Diversitermes, while that of the minor soldier is similar to that of the intermediate soldier of Diversitermes.

Outline of head of major soldier in general similar to that of the soldier of Tenuirostritermes Holmgren, pear shaped and markedly broader posteriorly than anteriorly. In major soldier antennae with thirteen segments; in minor, twelve segments. Legs relatively short, length of hind tibia much less than length of head with nasus.

Postclypeus of *worker* nearly as long as half its width.

Small, light colored species.

Nasutitermes (Uniformitermes) barrocoloradensis, new species.

MAJOR SOLDIER (fig. 8, *a*, *b*).—Head pale yellowish, darker at margins and at base of nasus, nasus reddish, head pyriform, broadest posteriorly, gradually converging toward anterior margin, breadth more contrasting posteriorly and anteriorly than in minor soldier, markedly but not sharply constricted anteriorly, convex in profile except for slight depression about center of head, with numerous short hairs, but with few long hairs anteriorly and posteriorly. Nasus elongate, slender, cylindrical, slightly upturned at apex, with short hairs and a few long hairs at apex. Mandibles fairly short, sharp pointed, points turned outward.

Antennae light yellow-brown, of thirteen segments, with long hairs; third segment subclavate, slender, longer than second or fourth segments; fourth segment shorter than second; fifth longer and broader than fourth; sixth, seventh, and eighth segments longer and broader than fifth; segments becoming shorter toward apex; last segment slender and subelliptical.

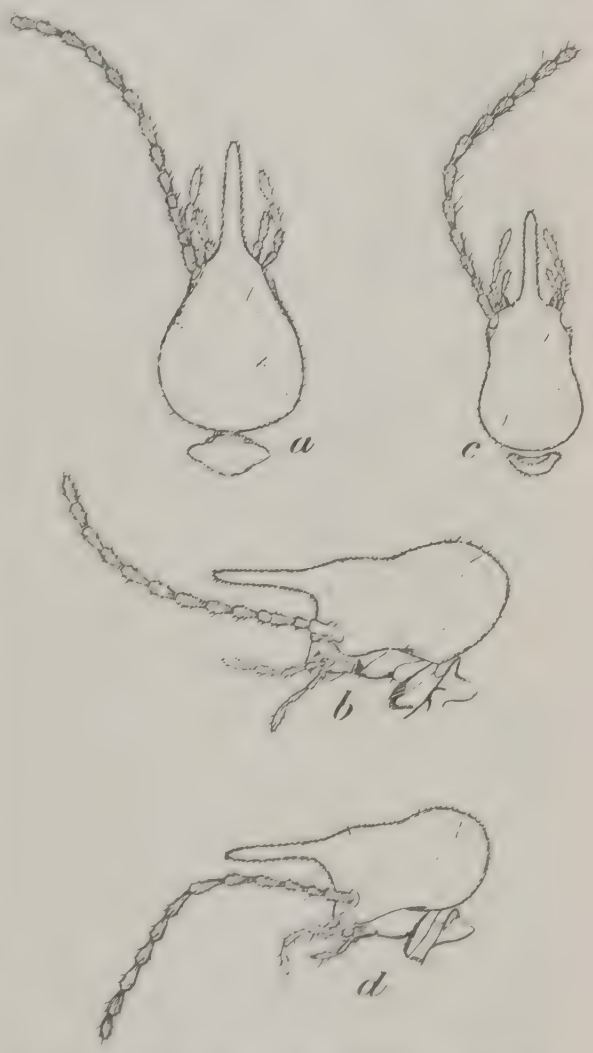


FIG. 8.—*Nasutitermes* (*Uniformitermes*) *barrocoloradensis*: *a*, Dorsal view of head and pronotum of major soldier; *b*, lateral view of same; *c*, dorsal view of head and pronotum of minor soldier; *d*, lateral view of same

Pronotum yellow, darker at anterior margin, saddle-shaped, with short hairs, broadly rounded anteriorly and posteriorly, sides slightly depressed posteriorly, slightly emarginate at anterior margin.

Legs with tinge of yellow, fairly elongate, slender, and with long hairs.

Abdomen dirty gray-white with tinge of yellow, tergites with short hairs and a row of long hairs at base of each tergite; cerci fairly prominent.

MEASUREMENTS (MAJOR SOLDIER).—Length of entire soldier: 3.1–3.3 mm. Length of head with nasus: 1.40–1.50 mm. Length of head to anterior margin: 0.90–1.00 mm. Length of nasus: 0.50–0.55 mm. Length of pronotum: 0.20 mm. Length of hind tibia: 1.10 mm. Width of head (where widest posteriorly): 0.70–0.80 mm. Width of head anteriorly: 0.40 mm. Width of pronotum: 0.40 mm.

MINOR SOLDIER (fig. 8, *c*, *d*).—Head pale yellowish, darker at margins and at base of nasus, nasus reddish, head somewhat pyriform, broadest posteriorly, gradually converging toward anterior margin, not as great a difference in breadth posteriorly and anteriorly as in major soldier, somewhat dumbbell shaped, markedly but not sharply constricted anteriorly, convex in profile except for slight depression about center of head, with numerous short hairs, but with few long hairs anteriorly and posteriorly. Nasus elongate, slender, cylindrical, slightly upturned at apex, with short hairs, a few long hairs at apex. Mandibles fairly short, sharp pointed, with points turned outward.

Antennae light yellow-brown, elongate, of twelve segments, with long hairs; third segment subclavate, longer than second but shorter than fourth; fifth about as long as fourth but broader; sixth longer than fifth; seventh slightly shorter than sixth; segments becoming shorter but broader toward apex; last segment more slender, subelliptical.

Pronotum pale yellow, anterior margin darker, saddle-shaped, with short hairs, margins broadly rounded anteriorly and posteriorly, sides slightly depressed posteriorly.

Legs with tinge of yellow, fairly elongate, slender and with long hairs.

Abdomen dirty gray-white with tinge of yellow, with short hairs on tergites and one row of long hairs at base of each tergite.

MEASUREMENTS (MINOR SOLDIER).—Length of entire soldier: 2.75–3.00 mm. (aver. 2.90 mm.). Length of head with nasus: 1.10–1.175 mm. Length of head to anterior margin: 0.75 mm. Length of nasus: 0.41 – 0.45 mm. Length of pronotum: 0.15 mm. Length of hind tibia: 0.90 mm. Width of head (where widest posteriorly): 0.50 mm. Width of head anteriorly: 0.40 mm. Width of pronotum: 0.25 mm.

Winged adult unknown.

TYPE LOCALITY.—Barro Colorado Island, Canal Zone, Panama.

Described from a large series of major and minor soldiers collected by T. E. Snyder with workers in a large colony at the type locality on February 26, 1924. Several colonies were found in decaying logs, with bark on, near the site of the new Tropical Research Station building.

TYPE, MAJOR SOLDIER.—Cat. No. 27270, U. S. National Museum; morphotype, minor soldier.

Mirotermes (Mirotermes) panamaensis Snyder.

DEÄLATED ADULT (MALE OR KING).—Head light yellow-brown, lighter posteriorly, suboval, longer than broad, not as broad as pronotum, with scattered long hairs, more numerous anteriorly, postclypeus light yellow, slightly bulging, twice as broad as long, prominently bilobed. Eyes black, fairly prominent, nearly round, separated from lateral margin of head by a distance less than their long diameter. Ocelli hyaline, suboval, separated from eyes by a distance hardly equal to the long diameter of an ocellus. Fontanelle, a hyaline narrow elliptical slit between middle of eyes.

Antennae light yellow-brown, of fifteen segments, with long hairs, becoming longer and broader toward apex; third segment very small, shorter than second or fourth segments, ringlike; fourth shorter than second segment; fifth ringlike, shorter than fourth; last segment elongate, slender, subelliptical.

Pronotum light yellow-brown, darker posteriorly, anterior and posterior margins nearly straight, slightly concave anteriorly and slightly emarginate posteriorly, sides rounded, sloping slightly to posterior margin, with long hairs.

Wing scale much shorter than pronotum.

Legs light yellow-brown, slender, with long hairs.

Abdomen with tergites brown, darker colored than head and pronotum, with fairly long hairs, lighter anteriorly; cerci prominent.

MEASUREMENTS.—Length of entire deälated adult (mature first-form male or king): 4.00 mm. Length of mature, deälated queens of the first form (with distended abdomens): 5.5–7.0 mm.; average, 6.76 mm. Length of head: 1.00 mm. Length of pronotum: 0.55 mm. Length of hind tibia: 0.70 mm. Diameter of eye: 0.16 mm. Width of head (at eyes): 0.85 mm. Width of pronotum: 0.95 mm. Width of abdomen of male: 1.40 mm. Width of abdomen of queens: 2.5–2.8 mm.; average, 2.65 mm.

King with abdomen slightly distended but queens with abdomens markedly distended and a flat quadrilateral shape.

Described from one male (mature, deälated, first-form adult or king) and nine females (mature, deälated, first-form adults with distended abdomens) collected with soldiers and workers in a very large colony in a decaying, fallen coconut palm tree trunk on the ground on a slight elevation near a mangrove swamp on February 19, 1924, at Largo Remo Island, Canal Zone, by T. E. Snyder and J. Zetek. These reproductive forms were in a small (about 7 inches wide) ovoid carton nest on the side of the interior of the log (Pl. 1, C and D).

M. (*Mirotermes*) *panamaensis* Snyder was described in 1923 from the soldier caste alone (fig. 9), the type locality being Barro Colorado Island, Canal Zone. This is the first time the sexual form or deälated adult has been collected; the winged form has not yet been found. The specimens are deposited in the collection of the United States National Museum.

LIST OF TERMITES OF THE CANAL ZONE AND NEAR-BY PANAMA

KALOTERMITIDAE: (100)

1. *Kalotermes tabogae* Snyder
2. *Kalotermes marginipennis* Latreille
3. *Kalotermes panamae* Snyder
4. *Neotermes holmgreni* Banks
[4] 5. *Kalotermes* (*Lobitermes*) *brevicollis* Banks
6. *Cryptotermes dudleyi* Banks (*Cryptotermes thompsonae* Snyder)
7. *Kalotermes* (*Lobitermes*) *longicollis* Banks
8. *Kalotermes* (*Glyptotermes*) *angustus* Snyder
[4] 9. *Kalotermes* (*Glyptotermes*) *emarginicollis* Snyder
[4] 10. *Kalotermes* (*Glyptotermes*) *barbouri* Snyder

RHINOTERMITIDAE: (5)

[4] 11. *Coptotermes niger* Snyder
[4] 12. *Leucotermes tenuis* Hagen
13. *Leucotermes convexinotatus* Snyder
14. *Prorhinotermes molinoi* Snyder
15. *Rhinotermes longidens* Snyder

TERMITIDAE: (21)

[4] 16. *Cylindrotermes nordenskiöldi* Holmgren
17. *Amitermes medius* Banks
[4] 18. *Amitermes beaumonti* Banks
[4] 19. *Cornitermes acignathus* Silvestri
20. *Armitermes* (*Armitermes*) *armigera* Motschulsky
[4] 21. *Armitermes* (*Armitermes*) *chagresi* Snyder
22. *Armitermes* (*Rhynchotermes*) *perarmatus* Snyder
[4] 23. *Nasutitermes cornigera* Motschulsky
[4] 24. *Nasutitermes pilifrons* Holmgren
[4] 25. *Nasutitermes ephratae* Holmgren
[4] 26. *Nasutitermes columbicus* Holmgren

FIG. 9.—*Mirotermes* (*Mirotermes*) *panamaensis*: Dorsal view of head and pronotum of soldier

[4] 27. *Nasutitermes* (*Uniformitermes*) *barrocoloradensis* Snyder
28. *Nasutitermes* (*Subulitermes*) *zeteki* Snyder
[4] 29. *Nasutitermes* (*Obtusitermes*) *biformis* Snyder
30. *Anoplotermes gracilis* Snyder
[4] 31. *Anoplotermes parvus* Snyder
[4] 32. *Eutermes debilis* Heer=*arboreus* Emerson
[4] 33. *Eutermes exiguus* Hagen
34. *Mirotermes* (*Mirotermes*) *hispaniolae* Banks
[4] 35. *Mirotermes* (*Mirotermes*) *panamaensis* Snyder
[4] 36. *Orthognathotermes wheeleri* Snyder

[4] Occurring on Barro Colorado Island, C. Z., the site of the Panama laboratory of the Institute for Research in Tropical America; there occur here twenty species and fifteen genera or subgenera.

THE SHAPE AND WEIGHT OF EGGS IN RELATION TO THE SEX OF CHICKS IN THE DOMESTIC FOWL [1]

By M. A. Jull, *Senior Poultryman*, and J. P. Quinn, *Poultryman, Bureau of Animal Industry, United States Department of Agriculture*

REVIEW OF PREVIOUS WORK

A study of the causes that might affect the production of one sex or the other in the domestic fowl should have an important bearing on the practical aspects of the poultry industry and on current theoretical questions concerning sex determination. The senior author [2] has made a number of observations concerning the sex-ratio situation in the domestic fowl, including the following:

1. There is no apparent correlation between egg weight and sex ratio.
2. There is no apparent correlation between yolk weight and sex ratio.
3. There is no apparent correlation between yolk water-content and sex ratio.

The authors are not aware of any observations having been made concerning the relationship between the shape of the egg and the sex of the chick hatched from it. A solution of this problem is important in order that the result may be added to results already determined and also because of the prevailing opinion among many practical poultrymen that long eggs usually produce male chicks. The poultry office of the Animal Husbandry Division of the United States Department of Agriculture is continually receiving letters either stating that the longer eggs produce more males than the shorter ones or inquiring if such is not the case. The data presented in this study serve as an answer.

The data in this study also deal with the relationship between the weight of the egg and the sex of the chick hatched from it. It has been stated above that the senior author found no correlation to exist between egg weight and sex ratio. This observation was based upon the results obtained in determining the sex of chicks from eggs laid during the first year of production by 45 Barred Plymouth Rock pullets mated to Brown Leghorn males. The sex was determined of all embryos that died from the twelfth day to hatching time as well as of all chicks that hatched. There was found to be no correlation between weight of egg and sex of chick.

On the other hand, in another study the senior author [3] found a significant difference between the weights of eggs from which males were obtained and those from which females were obtained. This was based upon data used in a determination of growth rates in the sexes of pure-bred Barred Plymouth Rock chicks. The only egg weights taken into consideration were those producing the chicks on which growth rates were determined, the records for analysis being selected at the conclusion of the growth period. The weights of the eggs in which embryos died and the weights of the eggs producing chicks which died prior to the termination of the period of growth studied were not considered. From the sex standpoint there may have been a differential prenatal or a differential postnatal mortality, or both. The weights of the eggs producing the chicks which completed the growth test showed that the eggs which produced males were heavier than the eggs which produced females, 52.11 ± 0.41 and 50.05 ± 0.53 gm. The difference in mean weights is 2.06 ± 0.67 gm., a difference which is slightly more than three times its probable error and therefore barely significant.

The case in which there was established no correlation between egg weight and chick sex was with eggs laid throughout the first year of production

[1] Received for publication June 23, 1924—issued January, 1925.

[2] Jull, M. A. The relation of antecedent egg production to the sex ratio in the domestic fowl. Jour. Agr. Research, XXVIII: 199–224, 1924.

[3] Jull, M. A. Differential sex growth curves in barred plymouth rock chicks. Sci. Arg. 4: 58–65, illus. 1923.

of each of 45 pullets, while the case in which there was a difference in the mean weights of eggs producing the two sexes was with eggs laid during the normal hatching season.

In view of the apparently different results obtained in the latter case, it was thought advisable to make additional studies, particularly with eggs laid during the normal hatching season.

EGG SHAPE IN RELATION TO CHICK SEX

The eggs of 24 Barred Plymouth Rock pullets laid between the middle of February and the last of April were measured carefully at the time of laying. The length and maximum breadth of each of 990 eggs were measured in millimeters, the measurements being recorded to hundredths of a millimeter. The sex of the chicks was determined by dissection at hatching time.

In Table I is shown the frequency distribution in terms of length in millimeters of the eggs producing males and those producing females. Of the 990 eggs, 512 produced males and 478 produced females. The mean length of the eggs producing males is 55.31 ± 0.06 mm. and the mean length of the eggs producing females is 55.42 ± 0.07 mm. The eggs producing females have a mean length slightly greater than that of the eggs producing males, but the difference is not at all significant since it is only 0.11 ± 0.09, as shown in Table VIII. The variation in length, both absolutely and relatively, is quite small in each group of eggs producing males and females. In Table II the production of each pullet is considered separately and there is not a single case in which the mean length of eggs producing males and those producing females is significantly different. Moreover, among the 24 pullets there are 13 in which the mean length of eggs producing males is greater than the mean length of eggs producing females and 11 in which the reverse is the case. It can be said, therefore, that there is no relation between length of egg and the sex of the chick hatched from it.

The shape of an egg is determined by considering the length and breadth in relation to each other. The length-breadth index was used as a measure of shape and was obtained by dividing one hundred times the breadth by the length. A long and narrow egg has a relatively low index, while a short and broad egg has a high index. Based on the assumption that long eggs usually produce a preponderance of males, it should be expected that eggs producing males would have a lower index than eggs producing females. The frequency distribution of shape index and the relative mean indexes of eggs producing males and females, respectively, are shown in Table III. The mean index of eggs producing males is 75.17 ± 0.09 and the mean index of eggs producing females is 75.09 ± 0.10, the difference with its probable error being 0.08 ± 0.13, as shown in Table VIII.

There is very little difference in respect to the relative and absolute amounts of variability between the groups of eggs producing the two sexes. In Table IV the mean indexes of eggs producing males and females, respectively, are shown for each pullet and in no case is the difference in the mean index significant. It is apparent, therefore, that there is no correlation between the relative length of an egg and the sex of the chick hatched from it. In other words, long and narrow eggs having relatively low indexes as compared with short, broad ones, are not likely to produce male chicks in any greater proportion than eggs with relatively high indexes.

EGG WEIGHT IN RELATION TO CHICK SEX

Eggs from two different sources were used in this study of egg weight in relation to the sex of chicks. In the first case eggs were obtained from 153 Barred Plymouth Rock females mated to Rhode Island Red males and in the second case from 58 Rhode Island Red females mated to Rhode Island Red males. The weights of 418 and 226 eggs in the former and latter cases, respectively, are taken into consideration, making a total of 644 eggs. The eggs were weighed daily as laid, the weights being recorded to hundredths of a gram.

TABLE I.—*Frequency distribution of egg length and the relative mean length of eggs producing males and females, respectively*

Egg length	Eggs producing—		
	Males	Females	Total
Mm.			
50.00 to 50.99	6	8	14
51.00 to 51.99	18	15	33
52.00 to 52.99	25	25	50
53.00 to 53.99	68	65	133
54.00 to 54.99	77	75	152
55.00 to 55.99	87	83	170
56.00 to 56.99	86	80	166
57.00 to 57.99	65	61	126
58.00 to 58.99	47	37	84
59.00 to 59.99	18	16	34
60.00 to 60.99	15	10	25
61.00 to 61.99		3	3
Total	512	478	990
Mean length	55.31±0.06	55.42±0.07	55.37±0.04
Standard deviation	2.18±0.05	2.17±0.05	2.17±0.03
Coefficient of variability	6.81±0.14	7.02±0.12	3.92±0.06

TABLE II.—*Mean egg length per bird for eggs producing males and females respectively*

Pullet No.	Eggs producing males		Eggs producing females		Difference
	Number	Mean length	Number	Mean length	
		Mm.		*Mm.*	*Mm.*
1	16	54.83±0.18	9	54.36±0.14	0.47±0.23
2	14	54.32±0.16	14	54.64±0.13	0.32±0.21
3	18	54.96±0.16	16	55.21±0.15	0.15±0.22
4	11	55.06±0.21	15	55.18±0.19	0.12±0.28
5	13	57.21±0.18	16	56.84±0.12	0.33±0.22
6	14	54.30±0.20	14	54.27±0.17	0.03±0.26
7	17	56.58±0.17	13	57.21±0.21	0.63±0.27
8	26	55.42±0.16	20	55.30±0.12	0.12±0.20
9	25	55.13±0.14	24	55.25±0.16	0.12±0.21
10	29	54.72±0.22	18	53.84±0.18	0.88±0.30
11	17	54.34±0.16	17	54.94±0.17	0.60±0.23
12	36	54.87±0.13	35	54.74±0.22	0.13±0.25
13	19	58.62±0.19	16	57.93±0.22	0.69±0.29
14	36	57.79±0.23	36	58.40±0.14	0.16±0.27
15	20	54.90±0.14	38	54.69±0.11	0.31±0.18
16	14	56.67±0.18	15	57.01±0.15	0.34±0.23
17	18	55.39±0.17	14	55.25±0.21	0.14±0.27
18	32	55.08±0.13	12	55.17±0.14	0.09±0.16
19	25	56.72±0.12	25	56.55±0.19	0.17±0.22
20	27	54.37±0.14	26	54.57±0.20	0.20±0.24
21	35	55.11±0.18	36	54.98±0.23	0.13±0.29
22	8	55.44±0.21	11	55.26±0.22	0.18±0.30
23	27	57.23±0.11	17	57.01±0.14	0.22±0.18
24	15	54.86±0.15	21	55.23±0.18	0.37±0.23
Total	512	55.31±0.06	478	55.42±0.07	0.11±0.09

TABLE III.—*Frequency distribution of shape index and the relative mean indexes of eggs producing males and females, respectively*

Length-breadth index	Eggs producing—		
	Males	Females	Total
63.00 to 63.99		1	1
64.00 to 64.99			
65.00 to 65.99	1	2	3
66.00 to 66.99	3	3	6
67.00 to 67.99	5	5	10
68.00 to 68.99	9	10	19
69.00 to 69.99	16	16	32
70.00 to 70.99	20	22	42
71.00 to 71.99	31	25	56
72.00 to 72.99	40	37	77
73.00 to 73.99	49	53	102
74.00 to 74.99	62	53	115
75.00 to 75.99	70	66	136
76.00 to 76.99	59	56	115
77.00 to 77.99	57	51	108
78.00 to 78.99	35	38	73
79.00 to 79.99	29	16	45
80.00 to 80.99	12	12	24
81.00 to 81.99	7	5	12
82.00 to 82.99	3	4	7
83.00 to 83.99	3	0	3
84.00 to 84.99	1	3	4
Total	512	478	990
Mean index	75.17±0.09	75.09±0.10	75.13±0.07
Standard deviation	3.20±0.06	3.28±0.07	3.24±0.05
Coefficient of variability	4.26±0.09	4.37±0.09	4.31±0.06

TABLE IV.—*Mean egg length-breadth index per bird for eggs producing males and females, respectively*

Pullet No.	Eggs producing males		Eggs producing females		Difference
	Number	Mean length-breadth index	Number	Mean length-breadth index	
1	16	75.38±0.56	9	73.83±0.62	1.55±0.83
2	14	76.47±0.37	14	75.74±0.41	0.73±0.55
3	18	75.22±0.77	16	77.59±0.38	2.37±0.86
4	11	73.62±0.54	15	71.69±0.60	1.93±0.81
5	13	77.76±0.41	16	77.48±0.27	0.28±0.49
6	14	76.33±0.32	14	77.28±0.40	0.95±0.51
7	17	78.79±0.24	13	78.02±0.36	0.77±0.43
8	26	81.03±0.28	20	80.82±0.22	0.21±0.36
9	25	74.03±0.19	24	74.47±0.27	0.43±0.33
10	29	76.15±0.16	18	77.00±0.14	0.85±0.21
11	17	75.13±0.31	17	74.20±0.24	0.93±0.39
12	36	76.59±0.29	35	77.16±0.14	0.57±0.32
13	19	72.25±0.48	16	71.94±0.71	0.31±0.86
14	36	76.05±0.22	36	75.87±0.13	0.18±0.25
15	20	72.74±0.26	38	73.31±0.15	0.57±0.30
16	14	73.42±0.27	15	72.67±0.29	0.75±0.40
17	18	74.13±0.23	14	73.13±0.33	1.00±0.40
18	32	74.51±0.14	12	74.30±0.41	0.21±0.43
19	25	75.93±0.20	25	77.01±0.26	1.08±0.33
20	27	69.55±0.18	26	69.64±0.15	0.09±0.23
21	35	70.94±0.12	36	70.79±0.17	0.15±0.21
22	8	77.92±0.49	11	77.20±0.38	0.72±0.62
23	27	77.33±0.19	17	76.49±0.31	0.84±0.36
24	15	74.46±0.27	21	74.01±0.16	0.45±0.31
Total	512	75.17±0.09	478	75.09±0.10	0.08±0.13

TABLE V.—*Frequency distribution and mean weights of eggs producing male and female chicks, respectively, that hatched*

BARRED PLYMOUTH ROCK FEMALES MATED TO RHODE ISLAND RED MALES

Egg weight	Eggs producing—		
	Males	Females	Total
Grams			
47.00 to 47.99		2	2
48.00 to 48.99	1	1	2
49.00 to 49.99	3	0	3
50.00 to 50.99	1	0	1
51.00 to 51.99	2	2	4
52.00 to 52.99	2	4	6
53.00 to 53.99	4	5	9
54.00 to 54.99	19	14	33
55.00 to 55.99	16	16	32
56.00 to 56.99	14	13	27
57.00 to 57.99	26	17	43
58.00 to 58.99	22	15	37
59.00 to 59.99	20	16	36
60.00 to 60.99	13	15	28
61.00 to 61.99	10	8	18
62.00 to 62.00	6	7	13
63.00 to 63.99	10	8	18
64.00 to 64.99	6	2	8
65.00 to 65.99	9	3	12
66.00 to 66.99	3	3	6
67.00 to 67.99	2	2	4
68.00 to 68.99	1	4	5
Total	190	157	347
Mean weight	58.64±0.19	58.53±0.21	58.58±0.14
Standard deviation	3.83±0.13	3.92±0.15	3.95±0.10
Coefficient of variability	6.53±0.22	6.70±0.25	6.74±0.17

TABLE VI.—*Frequency distribution and mean weights of eggs producing male and female chicks, respectively, that died in shell at hatching time*

BARRED PLYMOUTH ROCK FEMALES MATED TO RHODE ISLAND RED MALES

Egg weight	Eggs producing—		
	Males	Females	Total
Grams			
49.00 to 49.99		1	1
50.00 to 50.99	1	3	4
51.00 to 51.99	0	1	1
52.00 to 52.99	1	0	1
53.00 to 53.99	2	2	4
54.00 to 54.99	2	1	3
55.00 to 55.99	6	4	10
56.00 to 56.99	4	3	7
57.00 to 57.99	2	6	8
58.00 to 58.99	2	2	4
59.00 to 59.99	4	10	14
60.00 to 60.99	1	1	2
61.00 to 61.99	1	1	2
62.00 to 62.99	0	1	1
63.00 to 63.99	2	2	4
64.00 to 64.99	1	2	3
65.00 to 65.99	1	0	1
66.00 to 66.99		0	0
67.00 to 67.99		0	0
68.00 to 68.99		1	1
Total	30	41	71
Mean weight	57.56±0.44	57.82±0.43	57.71±0.31
Standard deviation	3.56±0.31	4.09±0.30	3.92±0.22
Coefficient of variability	6.18±0.54	7.07±0.53	6.77±0.38

TABLE VII.—*Frequency distribution and mean weights of eggs producing male and female chicks, respectively, that hatched*

RHODE ISLAND RED FEMALES MATED TO RHODE ISLAND RED MALES

Egg weight	Eggs producing—		
	Males	Females	Total
Grams			
46.00 to 46.99	----------	1	1
47.00 to 47.99	----------	0	0
48.00 to 48.99	----------	0	0
49.00 to 49.99	1	1	2
50.00 to 50.99	3	0	3
51.00 to 51.99	4	2	6
52.00 to 52.99	1	5	6
53.00 to 53.99	8	4	12
54.00 to 54.99	10	9	19
55.00 to 55.99	14	13	27
56.00 to 56.99	6	8	14
57.00 to 57.99	9	6	15
58.00 to 58.99	8	10	18
59.00 to 59.99	13	10	23
60.00 to 60.99	13	8	21
61.00 to 61.99	7	5	12
62.00 to 62.99	11	7	18
63.00 to 63.99	2	7	9
64.00 to 64.99	5	2	7
65.00 to 65.99	3	2	5
66.00 to 66.99	1	0	1
67.00 to 67.99	3	0	3
68.00 to 68.99	0	0	0
69.00 to 69.99	1	0	1
70.00 to 70.99	1	2	3
Total	124	102	226
Mean weight	58.06±0.26	57.29±0.27	57.75±0.19
Standard deviation	4.28±0.18	4.11±0.19	4.21±0.13
Coefficient of variability	7.37±0.31	7.17±0.33	7.29±0.23

TABLE VIII.—*Mean lengths, mean length-breadth indexes, and mean weights of eggs producing males and females, respectively*

Item	Eggs producing—		Difference
	Males	Females	
Mean length mm	55.31±0.06	55.42±0.07	0.11±0.09
Mean length-breadth index	75.17±0.09	75.09±0.10	0.08±0.13
Mean weight Rock × Red, hatched gm	58.64±0.19	58.53±0.21	0.11±0.28
Mean weight Rock × Red, died in shell do	57.56±0.44	57.82±0.43	0.26±0.61
Mean weight Rock × Red, total do	58.49±0.17	58.45±0.19	0.04±0.25
Mean weight Red × Red, hatched do	58.06±0.26	57.29±0.27	0.77±0.37
Mean weight, all eggs do	58.24±0.14	58.01±0.16	0.23±0.21

Of the 418 eggs from the Barred Plymouth Rock females, 347 hatched and 71 died in shell at hatching time. Distinguishing the sex of the chicks at hatching time was readily done, since the sex-linked barring pattern of the female parents is transmitted to the sons only. The male chicks, therefore, had the characteristic white spot on the back of the head and yellow shanks. The female chicks were solid black in down color and had very dark-colored or black shanks. Table V shows for the 347 chicks that hatched, the frequency distribution and the mean weights of the eggs producing males and females, respectively. The mean weight of the 190 eggs that produced males is 58.64 ± 0.19 gm. and the mean weight of the 157 eggs that produced females is 58.53 ± 0.21 gm. The difference in mean weight, with its probable error, is 0.11 ± 0.28 and, therefore, is of no significance. There is very little difference in respect to the amount of variability, either relatively or absolutely, in the groups of eggs producing the two sexes. The same situation prevails in respect to the 71 eggs whose chicks died in shell at hatching time,

as shown in Table VI. The mean weight of the 30 eggs producing males is 57.56 ± 0.44 gm. and the mean weight of the 41 eggs giving rise to females is 57.82 ± 0.43 gm. The difference, with its probable error, is 0.26 ± 0.61 and, therefore, is not significant. When the weights of the eggs that hatched and the weights of the eggs whose chicks died in shell at hatching time are taken into consideration, it is found that the mean weight of the eggs producing males is 58.49 ± 0.17 gm. and the mean weight of the eggs producing females is 58.45 ± 0.19 gm. as shown in Table VIII. The difference, with its probable error, is 0.04 ± 0.25 and, therefore, is of no significance.

From the Rhode Island Red females there were secured 226 eggs that hatched and on which the sex of the chicks was later recorded. The frequency distribution and mean weights of the eggs producing males and females, respectively, are shown in Table VII. The mean weight of the eggs producing males is 58.06 ± 0.26 gm. and the mean weight of the eggs producing females is 57.29 ± 0.27 gm. The difference, with its probable error, as shown in Table VIII, is 0.77 ± 0.37 and, therefore, is not significant. The relative and absolute amounts of variability in both groups are quite small, as shown in Table VII.

The mean weights of all eggs from the Barred Plymouth Rock and Rhode Island Red females producing males and females, respectively, are shown in Table VIII. The mean weight of all eggs producing males is 58.24 ± 0.14 gm. and the mean weight of all eggs producing females is 58.01 ± 0.16 gm. The difference, with its probable error, is 0.23 ± 0.21 and, therefore, is not significant.

CONCLUSIONS

As a result of this study, the following observations apply to eggs produced during the normal hatching:

There is no correlation between the absolute length of egg and the sex of the chicks hatched from it.

There is no correlation between the relative length or shape of egg and the sex of the chick hatched from it.

There is no correlation between weight of egg and the sex of the chick hatched from it.

THE GROWING SEASON OF WESTERN YELLOW PINE [1]

By G. A. Pearson

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Observations at the Southwestern Forest Experiment Station, at Flagstaff, Ariz., over a period of 14 years have shown that the shoots of western yellow pine, *Pinus scopulorum*, begin to grow about the middle of May, elongate most rapidly in June, and practically finish their growth by July 1. The major height growth occurs during the driest month of the year, although the lower soil strata are always moist from the heavy winter precipitation. Curiously enough, shoot growth is not resumed during the summer rainy season except occasionally in very vigorous seedlings. The needles continue to grow well into August. Does diameter growth take place at the same time as height growth or later? This, unlike the elongation of stems and needles, is not subject to ocular observation.

During the summer of 1923 records of the diameter growth of two western yellow pines were maintained at the Southwestern Forest Experiment Station by means of MacDougal's dendrograph. Both of the trees are young and thrifty, one being 5.4 inches and the other 15.8 inches in diameter. The larger tree (No. 1) is in the edge of a group on a moderate slope with southeasterly exposure; the smaller one (No. 2) is more isolated and stands in a practically level situation near the north brow of a level bench about 500 feet distant.

For the benefit of those not familiar with the MacDougal dendrograph [2] its essential features are here briefly described without going into mechanical details. The instrument is held in place by a belt of wooden blocks drawn tightly around the trunk of the tree. Above this, supported by stiff wires, is a floating frame of *invar*, a metal of low temperature coefficient, encircling the trunk, but touching it only at two points. One of these points is an adjustable screw; the other, on the opposite side of the tree, is a quartz rod which slides freely in and out with every movement of the bark. On the outer end of this rod rests the short arm of an L-shaped lever whose long arm carries a pen. As the tree expands the pen is raised and as it contracts (a daily occurrence) the pen falls. The pen, in contact with a ruled sheet of paper on a revolving drum driven by clockwork, makes a continuous record of the most minute expansion or contraction of the tree.

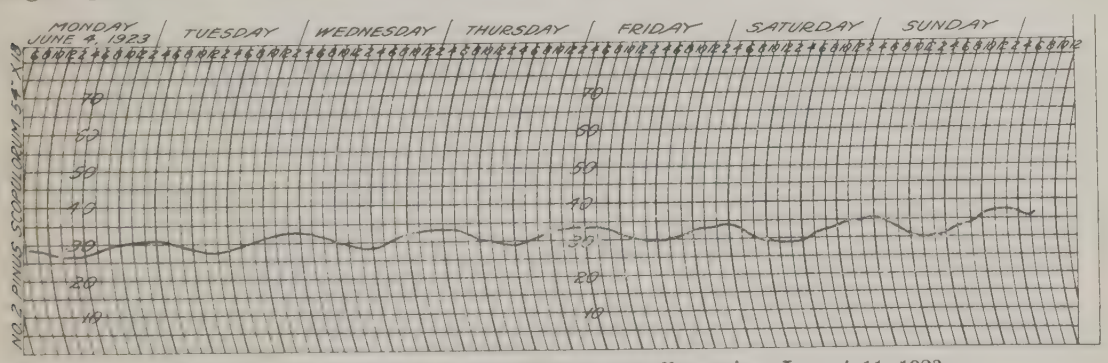

FIG. 1.—Dendrograph record of western yellow pine, June 4–11, 1923

Figure 1 shows the graph produced during a week of rapid growth. The strong diurnal movement does not reflect actual growth, but rather a swelling and shrinking which MacDougal has found to be intimately associated with the water content of the trunk. It will be noted that the maximum rise occurs at night or early in the morning, when transpiration is low. Actual growth or deposition of wood is indicated by the general upward trend of the graph. Thus, on June 4, the high point reads 31 mm. and on June 11, it reads 37 mm. Since the instru-

[1] Received for publication June 30, 1924—issued January, 1925.

[2] MACDOUGAL, D. T.—GROWTH IN TREES. 41 p., illus. Washington, D. C. 1921. (Carnegie Inst., Wash., Pub. 307.)

ment in this case was adjusted to give an amplification of 18, the growth amounts to about one-third of a millimeter in six days. A pronounced and sustained swelling, apart from actual growth, is recorded during long periods of rainy weather and a corresponding shrinkage in dry weather. Allowance must be made for these movements in computing current growth.

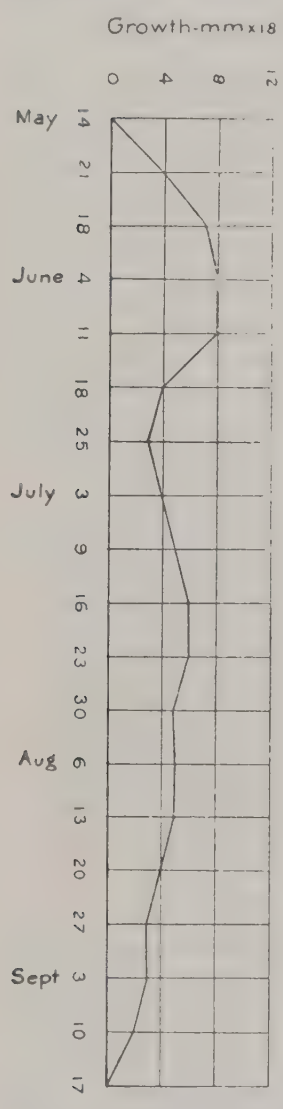

FIG. 2.—Diameter growth of western yellow pine, Southwestern Experiment Station, 1923. Tree No. 2 D. B. H., 5.4 inches. Total growth during season, 4.7 mm.

In this study the writer was interested primarily in determining at what time of the year diameter growth begins and ends. This is a matter of obvious importance in making periodic forest measurements with the object of ascertaining the annual rate of diameter growth. The relation of current growth to external factors such as temperature, moisture, and mechanical injuries offers an attractive field for research; but such a study would require vastly more attention than could be devoted to it by the writer at this time. Moreover, these relationships are being investigated by Doctor MacDougal, of the Carnegie Institution.

The instruments were installed on May 14. Tree No. 2 began to increase slightly in diameter on May 16, but No. 1 showed no signs of increase until June 1 (fig. 2). The reason for this difference is not apparent unless No. 2, because of its smaller size, was able to respond more quickly to the rising temperature. No. 1 continued to grow until about August 27, and No. 2 until about September 10. Thus the approximate periods of diameter accretion were 88 days for the larger tree and 117 for the smaller one. In both trees active growth was practically over by September 1.

It is of interest to compare the behavior of western yellow pine and other species in various localities of the Southwest. According to MacDougal, a Douglas fir, *Pseudotsuga taxifolia*, on the slopes of Pikes Peak, began growth before June 17; but a yellow pine, *Pinus ponderosa* (probably *scopulorum*), in the same locality, made no appreciable growth until August. The Douglas fir continued to grow until August 22, and the pine until September 4. *Pinus chihuahuensis* in the Santa Catalina Mountains of southern Arizona showed slight enlargements in April and the latter part of May, but made the major growth during the summer rainy season in August. *Pinus arizonica* and *Pinus strobiformis*, in the same locality, exhibited slight growth in June, followed by a cessation until the rains were in progress after the middle of July.

From the foregoing it appears that only in the case of yellow pine in northern Arizona and Douglas fir on Pikes Peak did active diameter growth begin in advance of the summer rains. Failure of yellow pine on Pikes Peak and of all the species in the Santa Catalina Mountains to grow in diameter during June is probably attributable to deficient moisture. This explanation, however, is not wholly adequate because in both of these regions, as well as in northern Arizona, the major height growth takes place prior to July 1, regardless of drought. Why the moisture should be insufficeint for diameter growth when it apparently is sufficient for height growth is not clear. This problem requires further investigation.

In studying growth by periodic measurement of standing trees, it is important that the trees, as far as diameter growth is concerned, be in a quiescent state at the time of each measurement. In Arizona and New Mexico, where growth plots have been remeasured every five years, it has been the custom to make these measurements in the fall, after September 1, assuming that the seasonal growth was finished by that date. The present study shows that this assumption was essentially correct. Investigations in southern Arizona and in Colorado indicate that in those regions, and probably throughout New Mexico, the species occurring below the Douglas fir type may also be measured in the spring and early summer until the summer rains are well in progress. In northern Arizona, however, measurements in the spring should not be made after May 15.

THE DIGESTIBILITY OF TEPARY BEANS[1]

By HARRY J. DEUEL

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The tepary bean, *Phaseolus acutifolius*, is a native North American crop plant and has long been grown in Sonora, Mexico, and in Arizona, by the Indian agriculturists. Bailey (*1*, *p. 462–463*)[2] states that the Papago and Pima Indians cultivated tepary beans from prehistoric times and "in all probability they formed one of the principal food crops of that ancient and unknown agricultural race." The plant forms a low, trailing bush, with many slender, diffuse branches which lie close to the soil. Tepary beans are distinctly a dry-land crop grown for the beans and rarely if ever for forage. The continuous growth with formation of seed pods, which is characteristic unless frost or disease interferes, is a disadvantage if the beans are grown in the moister regions of the United States. In the dry regions it appears that tepary beans will make a larger crop on less rain than any other known species of bean. There is therefore a large range of territory in California, Arizona, and New Mexico where their culture could be widely extended if a regular market demand existed for them.

The largest extension of tepary-bean culture probably took place in 1917, when California alone produced over 150,000 bushels. The market price was not satisfactory, and later crops have been considerably smaller.

Although somewhat smaller than navy beans the tepary resembles them very closely and may be readily mistaken for them. Forty-seven color types have been isolated by the Arizona Agricultural Experiment Station, but only the white tepary beans have entered bean-trade channels of the United States. Considerable attention has been given to tepary beans and their culture and use as food at the Arizona Agricultural Experiment Station (*4*) and the California Agricultural Experiment Station (*5*, *17*).

Much is known about beans and other common legumes as food, as a result of experiments carried on by many investigators. However, in the case of the tepary bean little information has been available with respect to food value beyond the analyses showing the chemical composition the fact that this bean is an important food crop where grown, that it is wholesome, well flavored, may be prepared in much the same ways as other dry beans, and that it is held to be a nutritious and sustaining food by those who have long used it. As in the case of most dry beans soaking is a necessary preliminary to cooking, or at least it shortens the cooking period. It is interesting to note that when soaked the skin of tepary beans wrinkles more quickly than does that of navy beans. In preparing tepary beans for the table Jaffa (*17*) recommends soaking them for 15 to 30 minutes and then draining and boiling in fresh water for about three hours.

Of beans, peas, and other dry legumes it may be said that they provide protein and carbohydrates (chiefly starch) in about equal amounts and in many cases some fat. That the range is rather wide is evident when one recalls that the soybean, like the peanut, is rich in fat, but when well ripened it contains no carbohydrate in the form of starch. In these respects the tepary bean obviously resembles the navy bean rather than the peanut or the soybean. With respect to the digestibility of the more common legumes, considerable information is available, chiefly as a result of investigations carried on by the United States Department of Agriculture and its collaborators.

A series of 70 experiments on the digestibility of navy beans, red kidney beans, and several varieties of cowpeas was made at the University of Tennessee (*31*). Generally speaking, the experiments show that the legumes were as well digested and assimilated as are the coarser cereal products, and that in some instances the digestibility was as great as that of the finer grades of flour. The investigations as a whole, it is stated, demonstrate the

[1] Received for publication June 21, 1924—issued January, 1925.

[2] Reference is made by number (italic) to "Literature cited," pp. 205-208.

important place which legumes may fill in the diet as economical and palatable sources of protein, though later studies have shown that most legume proteins can not be considered complete. However, a diet containing meat, milk, eggs, and similar foods would supply this deficiency.

Digestion experiments have been made in connection with the food work of the United States Department of Agriculture with soybeans and with peanuts (*15*) cooked until soft in a household pressure cooker. The legumes formed the principal part of a simple mixed diet. The experiments show that steam-cooked peanuts were well assimilated, the coefficient of digestibility of the protein being 79.9 per cent. Large quantities of these legumes were consumed throughout the experiments, no physiological disturbances being noted. The report also points out that as regards nutritive and biological value, there is evidence to justify the belief that soybeans and peanuts are especially valuable as food in comparison with other legumes which had been similarly studied. The literature of this phase of the subject is summarized in the bulletins cited.

EXPERIMENTAL METHODS

The experimental methods used with tepary beans were those followed in digestion experiments conducted by the United States Department of Agriculture and described in earlier publications (*2, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29*).

The subjects were men apparently in good health and well instructed in the experimental routine. The necessary analyses of food and feces were made by standard methods. The tepary beans used in the experiments were obtained through the courtesy of one of the correspondents of the department. The beans were prepared by soaking over night and then cooking for an hour under 15 pounds pressure. The beans were eaten with a basal diet of bread, butter, fruit, and sugar. The results of the five experiments are summarized in Table I.

TABLE I.—*Summary of digestion experiments with tepary beans in a simple mixed diet*

Experiment No.	Digestibility of entire ration				Estimated digestibility of tepary bean protein	Estimated digestibility of tepary bean carbohydrate
	Protein	Fat	Carbohydrate	Ash		
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
901	86.3	93.8	98.1	81.4	84.8	97.9
902	78.7	92.3	98.2	75.6	74.4	100.0
903	80.4	94.2	98.1	77.3	74.6	98.4
904	73.4	91.9	96.7	65.9	67.1	96.4
905	82.8	93.7	97.9	72.9	78.7	97.4
Average	80.3	93.2	97.8	74.6	75.9	98.0

The subjects ate, on an average, 70 gm. of protein, 69 gm. of fat, and 394 gm. of carbohydrate, with an energy value of 2,475 calories, per man per day. The beans supplied on an average 40 gm. of protein and 99 gm. of carbohydrate per man per day. The protein of the beans was 76 per cent utilized, which agrees closely with the value of 78 per cent for navy and red kidney beans found by Wait (*31*), and 77.9 per cent found by Mendel and Fine (*30*) for navy-bean protein. The carbohydrates of the tepary beans were on an average 98 per cent digested, which represents almost complete utilization and is somewhat higher than the value of 96 per cent found by Wait for navy-bean carbohydrates. The subjects reported that they remained in their usual normal health throughout the experimental period.

SUMMARY

A study of the digestibility of tepary beans for purposes of comparison with similar legumes shows that tepary beans are well utilized by the body and are a valuable food.

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STRIPE RUST (PUCCINIA GLUMARUM) OF CEREALS AND GRASSES IN THE UNITED STATES [1]

By H. B. HUMPHREY, *Senior Pathologist in Charge of Cereal-Disease Investigations, Office of Cereal Investigations, Bureau of Plant Industry;* C. W. HUNGERFORD, *Pathologist, Idaho Agricultural Experiment Station, and Agent, Office of Cereal Investigations, Bureau of Plant Industry;* and A. G. JOHNSON, *Senior Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

Stripe rust was first reported from Europe by Schmidt (*22*) [3] in 1827. He described the fungus as *Uredo glumarum*. In 1894, Eriksson and Henning (*4, p. 197*) reported their discovery of the telial stage of the fungus, and transferred the species to the genus Puccinia. This, summarized, becomes *Puccinia glumarum* (Schm.) Erikss. and Henn. Prior to 1894, the fungus had been confused with *Puccinia rubigo-vera* DC., *P. dispersa* Erikss. and Henn., *P. graminis* Pers., and *P. coronata* Corda, specimens of which were deposited in several herbaria under the name *P. glumarum*. In North America, specimens 1471a and 1471b of the Ellis and Everhart Collection of North American Fungi, and referred to by them as *P. glumarum*, have since been found to have been erroneously classified. Likewise this is true of Ravenel's specimen No. 100 of his *Fungi Carolliniani exsiccati*. Rabenhorst's specimen No. 3214, collected in North America, the writers have not examined, but Sydow and Sydow (*23*) have questioned the correctness of its classification as *P. glumarum*. An examination of specimens in the herbarium of the New York Botanical Garden established the fact that *P. glumarum* was collected near Seattle and Everett, Wash., as long ago as June, 1892, when C. V. Piper reported it as *P. rubigo-vera* on *Elymus glaucus* Buckl. (*E. americanus* Vasey and Scribn.) and a month later on *Bromus carinatus hookerianus* (Thurb.) Shear.

Other American collections, made prior to 1915, are listed farther on under *Exsiccati*. It should be noted here, however, that an examination of rust specimens contained in the Arthur Herbarium, La Fayette, Ind., brought out the fact that in August, 1911, E. and E. T. Bartholomew collected *P. glumarum* on *Sitanion hystrix* (Nutt.) J. G. Smith. This collection was distributed as *Puccinia agropyri* No. 4611. In the specimen envelope, attached to the herbarium sheet, was found the following interesting note to Dr. Arthur in E. Bartholomew's handwriting:

> This differs so radically from what you have been calling *P. rubigo-vera* on this host as found in Iowa, Nebraska, and Kansas, that I am sending it to you for examination. Collected in an old, previously cultivated field where there is no hint of aecial infection. I have called it provisionally *P. glumarum* (Schm.) Erikss. and Henn., on *H. jubatum*, Rock River, Wyoming, August 24, 1911.

Thus it is seen how close Bartholomew came to definitely recognizing stripe rust in America in 1911.

Although stripe rust in the United States was not certainly identified as such until May, 1915, when it was

[1] Received for publication July 1, 1924—issued January, 1925. It was originally intended to make of this paper a comparative study on the occurrence of *Puccinia glumarum* in the United States and Europe. That part of the proposed paper relating to this rust in Europe was to have been contributed by the late Dr. F. Kølpin Ravn, of Denmark, as joint author. Owing to Dr. Ravn's untimely death this plan was abandoned. However, the notes made in the United States in connection with Dr. Ravn's trip here during 1915 are included because of their historic interest.

[2] The authors wish to make grateful acknowledgment to Dr. A. S. Hitchcock and Mrs. Agnes Chase for assistance in identifying the various grasses named in this paper, and to Dr. Ruth F. Allen, Mrs. Rose E. Gamble, and J. M. Shull for the preparation of certain of the illustrations. The authors are indebted to the Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, the New York Botanical Garden, Harvard University, the University of Minnesota, and the Washington and Purdue University Agricultural Experiment Stations for placing at their disposal the facilities of their several herbaria.

[3] Reference is made by number (italic) to "Literature cited," p 226–227.

specifically recognized by F. Kølpin Ravn, there exists in certain herbaria of the country convincing evidence of the existence of this rust in the United States for a period of at least 23 years prior to its discovery. Indeed, it is possible that stripe rust established itself in western North America centuries ago. Its occurrence on certain grasses indigenous to Alaska has led the writers to consider more fully the distribution of the rust by these hosts up and down the Pacific Coast and throughout the tributary intermountain territory.

During the summer of 1916 the writers made numerous collections of stripe rust on wheat and certain wild hosts in Skagit and San Juan Counties, Wash. Here they observed for the first time the occurrence of this rust on *Bromus pacificus* Shear and *B. sitchensis* Bong. Its occurrence on *B. marginatus* Nees had first been noted in June, 1915, at Pullman, Wash. Infected plants of *Hordeum jubatum*, *H. nodosum* L., and *Elymus glaucus* Buckl. have been collected frequently, the first collection on these hosts, as elsewhere noted, being that of E. and E. T. Bartholomew in 1911. *H. caespitosum* Scribn., *Agropyron spicatum* (Pursh) Rydb. and *A. violaceum* (Hornem.) Lange also are congenial hosts, and infected specimens have been collected by C. W. Hungerford.

All of the hosts of *P. glumarum* recorded in the foregoing paragraph are, according to Hitchcock, common to Alaska. Although the writers have no record of the occurrence of this rust on any of these grasses in Alaska, it seems likely that it may be found there. The fact that it has been observed on certain of these hosts in such isolated localities as San Juan and Vancouver Islands would tend to support the assumption that this rust occurs also in Alaska. And if it be present in Alaska, it seems not unreasonable to infer that it may have reached this continent from Siberia by way of Kamchatka and the Aleutian Islands, or during an earlier period, while the two continents were yet one.

COMMON NAME

The disease caused by *Puccinia glumarum* is commonly known among most British botanists and plant pathologists as "golden rust," though occasionally it is referred to as "yellow rust." In Germany and Austria it is known as *Gelbrost* (yellow rust), while in Sweden and Denmark it is called *Gulrost*. According to Ferraris (*5*), stripe rust is commonly known in France as *rouille jaune*, and in Italy as *ruggine striata del grano*. Thus it is noted that in all cases, excepting that of Italy, the common name is based solely on a color character.

To the writers it has seemed that the more descriptive common name, stripe rust, should be adopted, for certain other of the cereal rusts might quite properly be styled yellow or golden and thus prove confusing to the inexperienced student or observer. As an example of the need of greater attention to the descriptiveness of common names, the writers might cite their experience in attempting to determine just what rust is referred to by hundreds of voluntary crop reporters who record outbreaks of "rust," "red rust," "black rust," "yellow rust," etc. Many of the reports merely state "rust" and for this reason are of very doubtful, if, indeed, of any, value to the student interested in a specific rust.

For this disease, therefore, the writers propose the general adoption of the name stripe rust. This is believed to be preferable to the name yellow rust, not only for the reason that it is more definitely descriptive but also that it has the merit of at once enabling the observer to distinguish it from all other cereal rusts.

THE DISCOVERY OF STRIPE RUST IN THE UNITED STATES

Stripe rust was discovered in the United States and definitely determined as such by the late Dr. F. Kølpin Ravn, of Copenhagen. On May 25, 1915, while examining wheat fields near Sacaton, Ariz., he observed and collected the rust, recognizing it on the spot as *P. glumarum*. At the time of the discovery of this rust, the wheat near Sacaton was in the dough stage of development and no teliospores were observed. This would seem to indicate a possible late or delayed initial infection, for it should be added that in none of the plants observed was there evidence of more than a mild attack.

On the same day, May 25, 1915, one of the authors (Johnson) collected what was later identified as *Puccinia glumarum* on *Hordeum murinum* L. near Tehachapi, Kern County, Calif. Although a diligent search was made for the rust in the wheat fields of the lower Sacramento Valley and elsewhere in California during that year, it was not found. On June 10, of the same year, it was observed again at Corvallis, Oreg., by Dr. Ravn and others who noted its occurrence on Baart and

Chul wheat. Later in the same month it was found in abundance at Moro, Oreg., a district of relatively scant rainfall, and at other points in Washington and Idaho. These observations are referred to later.

DISTRIBUTION

Stripe rust is widely distributed throughout Europe, though its ravages are confined chiefly to the northern countries, that is, Great Britain, Sweden, Norway, Denmark, Belgium, France, Russia, and Austria. It is prevalent also in Egypt, Algeria, Japan, and India, but has not yet been reported from South America or from New Zealand or Australia.

Although authenticated reports of its occurrence in eastern Siberia are not at hand, its presence in western Siberia (Akmolinsk) and in Japan supports the belief that this rust may have become established on the mainland adjacent to Japan.

In North America, stripe rust thus far has been observed eastward to the Black Hills of South Dakota, northward as far as Duncan, British Columbia, and southward as far as Mexico City, Mex. Its distribution on this continent has not extended eastward beyond 103° W. longitude, notwithstanding the fact that in addition to the cultivated hosts, wheat, barley, and rye, it occurs naturally on at least 34 wild grasses, 13 of which extend over a wide range of territory east of the one hundred and third meridian. *Hordeum jubatum*, one of its most congenial hosts, now extends across the continent, yet the writers find it is not infected with *Puccinia glumarum* east of what may be designated the Rocky Mountain formation. Other wild-grass hosts, the distribution of which extends eastward beyond the Pacific and intermountain States, are *Agropyron spicatum*, *A. cristatum* (L.) Gaertn., *A. dasystachum* (Hook.) Scribn., *A. desertorum* (Link) Schult., *A. intermedium* (Host.) Beauv., *A. violaceum*, *Bromus rubens* L., *Elymus canadensis* L., *E. glaucus*, *E. striatus* Willd., *E. virginicus* L., *Hordeum nodosum*, *H. pusillum* Nutt., *Hystrix patula* Moench and *Sitanion hystrix*.

Just what factor or combination of factors has operated to prevent the march of stripe rust, if not simultaneously with, at least in the wake of, such hosts as have established themselves within the Mississippi Valley and beyond, has not yet been determined. It may be that it is slowly advancing eastward and that it is merely a matter of time before a visitation of this pest will befall the wheat fields of the Great Plains and the fertile prairies of Iowa, Minnesota, and Illinois. And yet, the writers' records show that *P. glumarum* certainly has been present in Wyoming since 1911. During the decade that has elapsed, there have been propitious rust years, but these have not extended the disease beyond the Black Hills in western South Dakota where, in August, 1919, it was observed by one of the authors (Johnson) on cultivated barley. In spite of repeated attempts to find it in this locality in subsequent seasons, it has not been observed on either cultivated or wild hosts.

The fact that the map (fig. 1) shows no recorded observation of this rust in either Nevada or New Mexico is not to be interpreted as evidence of the absence of *Puccinia glumarum* from those States. It is not unlikely that a more thorough and extensive survey, such, for example, as has been conducted in Oregon and Washington, would have resulted in its discovery in both Nevada and New Mexico.

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Petrak, *Flora Bohemiae et Moraviae exsiccata.* 376./a (II, *Secale cereale*,

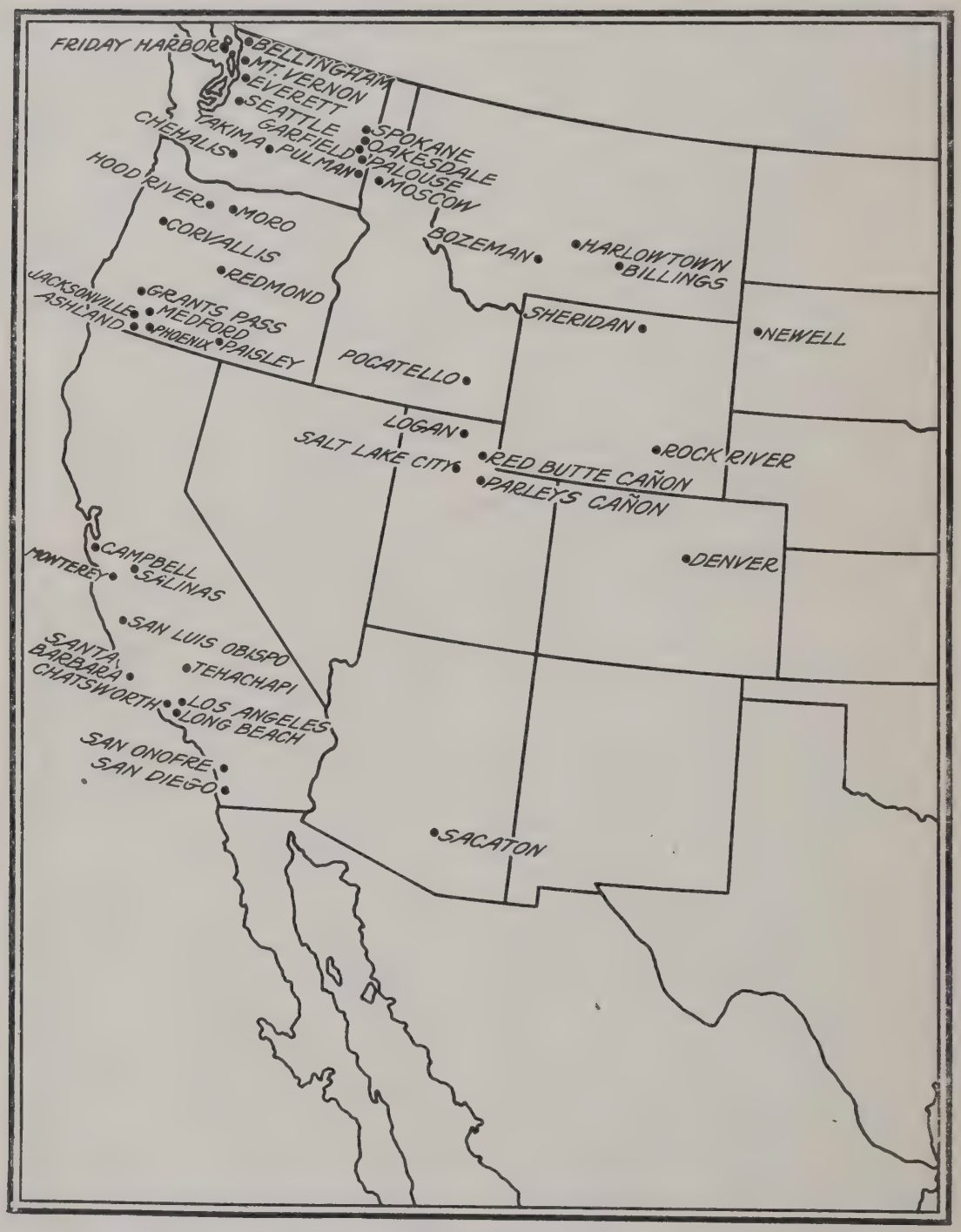


FIG. 1.—Map showing present known distribution of stripe rust in the United States

Boehmen, 6, 1894); 376./b (II, *Calamagrostis epigeios*, Moravia, 9–1912).

Fungi Eichleriani. 104 (*Hordeum vulgare*, Moravia, May, 1906); 105 (*Triticum aestivum*, Moravia, June, 1906).

Saccardo, *Mycotheca italica*. 1445 (II, III, *Secale cereale*, Italy, May, 1904).

Bucholtz and Bondarzew, *Fungi Rossiei exsiccati*, Ser. A, 163 (*Hordeum vulgare*, Russia, July, 1917).

Butler, E. J. (*Hordeum jubatum*, India, Feb., 1905).

Herb. Crypt. Ind. Orient. (II, *Hordeum vulgare*, India, April, 1904); (II, *Hordeum vulgare*, India, 1907); (II, III, *Hordeum vulgare*, India, March, 1903).

Westendorp et Wallays, *Herbier Cryptogamique*. 1845–1859. 231 ("leaves of cereals," Belgium. Issued as *Uredo rubigo-vera*).

Plantes cryptogames de France. 1476 (II, III, *Triticum* sp., France, 1845–1859. Issued as *Uredo glumarum*); 568 France, 1845–1859. Issued as *Uredo glumarum*).

Bartholomew, *North American Uredinales*. 1063 (III, *Hordeum jubatum*, Wyoming, Aug., 1911. Issued as *P. montanensis*); 2758 (III, *Agropyron smithii*, Colorado, Aug., 1916); 2435 (*Elymus glaucus*, California, June, 1919); 1755 (*Hordeum nodosum*, Colorado, Aug., 1916).

Bartholomew, *Fungi Columbiani*. 3763 (*Hordeum jubatum*, Wyoming, Aug., 1911. Issued as *P. montanensis*); 4369 (*Hordeum jubatum*, Montana, Aug., 1913. Issued as *P. rubigo-vera*); 4611 (*Sitanion elymoides*, Wyoming, Aug., 1911. Issued as *P. agropyri* (?)).

Garrett, *Fungi Utahenses*. 138 and 139 (II and III, *Elymus glaucus*, Utah, June, 1907. Issued as *P. rubigo-vera*); 191 (II and III, *Hordeum jubatum*, Utah, July, 1919. Issued as *P. rubigo-vera*); 192 (II and III *Hordeum pusillum* Utah, Aug., 1909. Issued as *P. rubigo-vera*).

Piper, *Washington Flora*. 41 (II, *Elymus Americanus*, Wash., June, 1892. Issued as *P. rubigo-vera*); 206 (*Bromus Hookerianus*, Wash., July, 1892. Issued as *P. rubigo-vera*).

Strand, *Flora of Montana*. 180 (II, *Hordeum jubatum*, Montana, Oct., 1914. Issued as *P. agropyri*).

Flora of Oregon. [Oregon Agricultural College.] 1385 (II, *Hordeum gussoneanum*, Oregon, June, 1914. Issued as *P. rubigo-vera*); 1423 and 1429 (*Sitanion hystrix*, Oregon, July, 1914. Issued as *P. agropyri*); 1596 (*Elymus glaucus*, Oregon, May, 1914. Issued as *P. agropyri*).

Ex Herb. Bethel. (*Hordeum jubatum*, Colorado, June, 1916. Issued as *P. agropyri*); (III, *Hordeum jubatum*, Colorado, July, 1916. Issued as *P. agropyri*).

Ex Herb. Holway. 3067 (*Hordeum jubatum*, Mexico, Oct., 1898. Issued as *P. rubigo-vera*).

ECONOMIC IMPORTANCE OF STRIPE RUST IN THE UNITED STATES

From present knowledge of the great economic importance of stripe rust in Europe, especially on wheat, and from the evidence of its virulence in the United States, particularly as noted on certain wheat varieties, it is not improbable that under optimum conditions its ravages might prove a serious menace to wheat culture on this continent.

It is more fully brought out later that the severity of the attacks on different organs of the wheat plant varies markedly with the variety. For example, in certain varieties of wheat, especially Chul, which is grown to a limited extent in the Western States, the attack is very severe on the glumes and kernels and rather indifferent on the leaves. In varieties where this type of infection occurs, the results are frequently serious. The rusted kernels become greatly shrunken, their quality generally lowered, and the yield consequently much reduced (Pl. 1, *c*).

Possibly the most far-reaching importance of the attack of the disease on the kernels is the connection that this type of infection may bear to carrying the fungus over from one crop to the next. The importance of the attack on the glumes and stalks is also referred to by Blaringhem (*3*) who records the greater injury from this type of attack.

In most varieties of wheat, stripe-rust infection is confined chiefly to the leaves. The abundance of such infection may vary greatly. In cases where it is severe, 85 to 100 per cent, there no doubt results noticeable and serious injury.

Because of its possible menace to wheat culture in the extensive and more humid wheatlands of the Mississippi Valley and of the Atlantic States, it is unquestionably important that more be learned about the life history of this rust; its present geographic distribution; its behavior and effects on different hosts and their activities; its seasonal

cycle; and the conditions which favor or inhibit its development. Above all, it is important that the utmost effort be made to confine this rust to its present known range in the Western States, for if one may judge of its possible behavior under conditions of greater humidity and more favorable temperature, from what is known of its ravages in Europe, its importance as a limiting factor in the production of wheat would scarcely be second to that of stem rust.

HOSTS

Not unlike *Puccinia graminis* Pers., *P. glumarum* is found on a relatively large number of both wild and cultivated Gramineae. Investigation has shown that definite specialized forms exist, such, for example, as those common to wheat and barley which, according to Eriksson and Henning (*4*), are sharply fixed. In Europe, where this rust has long been common, the following hosts have been recorded: *Calamagrostis epigeios* (L.) Roth, *Elymus arenarius* L., *Hordeum jubatum*, *H. vulgare* L., *Aegilops triuncialis* L. (*Triticum triunciale* (L.) Rasp.), *Secale cereale* L., *T. vulgare* Vill., *T. turgidum* L., *T. spelta* L., *T. repens* L. (*Agropyron repens* (L.) Beauv.), *T. polonicum* L., *T. giganteum* Roth, *T. durum* Desf., *T. monococcum* L., *T. dicoccum* Schrank, *T. desertorum* Fisch. (*Agropyron desertorum* Schult.), *T. compactum* Host., *T. caninum* Ledeb. (*Agropyron caninum* L.), and *Dactylis glomerata* L. Doubtless it has other hosts among the wild grasses of Europe, Asia, and Africa, such, for example, as other species of Agropyron, Bromus, Elymus, and Hordeum, but record of such is absent from the literature seen. One species particularly, *Agropyron cristatum* (L.) Gaertn., occurring naturally in Europe, and especially in Russia, has proved a most congenial host of *P. glumarum* in America, though it is not reported as such in Europe.

In North America, stripe rust thus far has been reported as occurring naturally on the following hosts: *Agropyron spicatum* (Pursh) Scribn. and Smith, *A. cristatum* (L.) Gaertn., *A. dasystachum* (Hook.) Scribn., *A. intermedium* (Host.) Beauv., *A. violaceum* (Hornem.) Lange, *A. lanceolatum* Scribn. and Smith (*A. riparium* J. G. Sm.?), *A. desertorum* Schult., *Bromus marginatus* Nees, *B. pacificus* Shear, *B. sitchensis* Trin., *B. carinatus* Hook. and Arn., *B. carinatus* v. *hookerianus* (Thurb.) Shear, *B. rubens L.*, *B. brizaeformis* Fisch. and Mey., *B. polyanthus* Scribn., *Elymus canadensis* L., *E. condensatus* Presl., *E. glaucus* Buckl., *E. macounii* Vasey, *E. striatus* Willd., *E. virginicus* L., *Hordeum caespitosum* Scribn., *H. jubatum* L., *H. gussoneanum* Parl., *H. murinum* L., *H. nodosum* L., *H. pusillum* Nutt., *H. vulgare* L., *Hystrix patula* Moench, *H. californica* (Boland.) Kuntze, *Phalaris paradoxa* L., *Sitanion jubatum* J. G. Smith, *S. hystrix* (Nutt.) J. G. Smith, *S. longifolium* J. G. Smith, *Secale cereale* L., *Triticum vulgare* Vill., *T. polonicum* L., *T. compactum* Host., *T. durum* Desf., *T. spelta* L., and *T. dicoccum* Schrank.

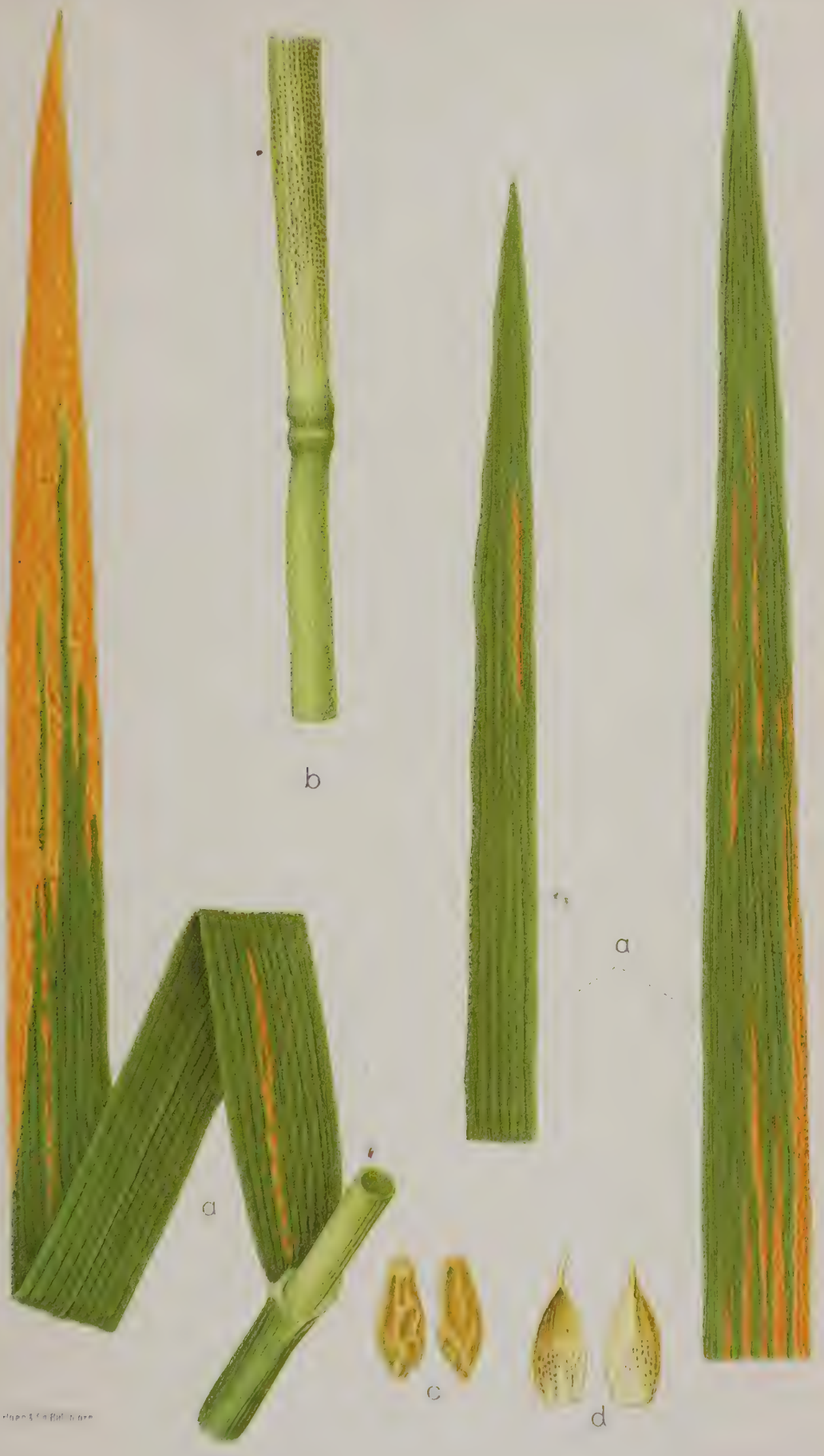
Thus far the writers have failed to note the occurrence of stripe rust on *Dactylis glomerata* in America, nor has it been possible for them to produce infection by artificial means, in spite of conditions highly favorable to inoculation and subsequent incubation. These facts would seem to indicate that there are inherent differences between the European and American forms of the rust or that the European and American forms of the host are not entirely identical.

DESCRIPTION OF STRIPE RUST

The aecial stage of *Puccinia glumarum* is unknown. Eriksson and Henning (*4*) report inoculation experiments with teliospores on various species of Boraginaceae, but in all instances they obtained only negative results. A further and more exhaustive study, however, may result in the discovery of the aecial host. Because one has not yet been found, is not sufficient justification for assuming its nonexistence, or that the rust, having formerly required such a host, may, under less exacting conditions, have lost its capacity for aecial development.

EXPLANATORY LEGEND FOR PLATE 1

a, Leaves with characteristic stripelike lesions; *b*, portion of culm showing fine stripes of telia; *c*, shriveled kernels from head of a severely rusted plant; *d*, ventral and dorsal views of glumes showing telia.


b
a
a
c
d

ON WHEAT

On the leaves of adult wheat plants, the uredinia develop in end-to-end series between the vascular bundles, forming long stripes, sometimes extending throughout the length of the leaf. Many of these, developing side by side, seem to coalesce, thus completely transforming one or the other, or both, surfaces of the leaf to a mass of uredinia. Most commonly the rust occurs on the leaf blades, but it appears not infrequently also on the leaf sheaths, and in certain peculiarly susceptible varieties of wheat the glumes, awns, and kernels are severely attacked. On the leaf blades of adult plants, the uredinia are irregularly scattered and strikingly characteristic as to form and color. In general appearance they are much the same on both sides of the leaf. They are narrowly oblong to linear in form, sharply delimited laterally and less so at the ends (Pl. 1, *a* and *b*). Individually, they vary in extent from about 1 to 3 mm. in width and from 10 to 110 mm. in length. When urediniospores alone are present, the color of the stripelike lesions is a reddish orange yellow when the uredinia are open and lemon yellow when they are not open. Mikado orange to capucine yellow, according to Ridgway (*21*).

On the more susceptible varieties, where uredinia are numerous on the same leaf blade, the attacked areas not uncommonly coalesce, either laterally, or end-to-end, or both, to such an extent that practically the entire leaf blade, or large portions of it, may be involved. On the sheaths, similar stripelike lesions occur, but they usually are fewer and less conspicuous. On the glumes, the attack is strikingly characteristic, especially on certain varieties of wheat which are apparently more susceptible than others in these organs.

When the wheat heads are normally green, those attacked by the rust take on varying shades of yellowish green because of deficient chlorophyll in affected glumes. The number and distribution of glumes attacked on individual wheat heads may vary considerably, from a single glume to several widely scattered. In those heads where infection is most general and pronounced, all the glumes may be rusted. The uredinia and telia develop almost entirely on the ventral side of the glume, thus causing the color and general appearance of rusted heads to vary according to the severity of the infection. In milder cases only slight discoloration is noticeable. Instances of severe infection are characterized by marked discoloration and an abundance of free urediniospores which accumulate in such numbers as to form a yellow powdery mass on the inner surface of the glumes and over the developing kernel (Pl. 1, *d*). Likewise, the kernels vary markedly in appearance, depending on the severity of attack. When there are but few uredinia on the glumes, there usually is little or no shrinkage of the kernels; but when the infection develops early and becomes severe, the damage is fully as marked as that resulting from a like infection of stem rust (Pl. 1, *c*). When such kernels are sectioned and examined with the microscope they usually are found infected to a greater or less extent. In such infected kernels, the spores are produced in characteristic pockets in the pericarp as shown in Figure 2, and also by Eriksson and Henning (*4*, *Pl. 9*).

In seedling infection, the uredinia appear in more or less rounded patches without any seeming tendency to form stripes. Indeed, in this stage of its development stripe rust does not differ markedly from orange leaf rust (*Puccinia triticina* Erikss.), except that in the latter the distribution of the uredinia is more general over the surface of the leaf, and the color of the spores in mass is darker yellow, more typically orange in color. This noticeable difference between the disposition and character of the uredinia on the leaves of adult and seedling plants is to be explained on purely anatomical grounds, as has been pointed out by Eriksson and Henning (*4*). Structurally, the seedling leaf is sparingly supplied with vascular tissue and is markedly succulent (fig. 3, *A*). The relative absence of vascular tissue contributes to the easy and general spread of the mycelium and the irregular eruption of the surface as outlets for the urediniospores. In the older or adult leaves, the vascular bundles check the spread of rust laterally and confine it to rather sharply defined areas, usually

many times longer than wide (fig. 3, B). Thus, if infection obtains near the tip or distal end of a leaf, the affected area widens as the fungus extends toward the base of the leaf.

The telial stage is not at all uncommon, especially later in the crop season. It may appear in any organ or structure of the host attacked by the uredinial stage of the rust, namely, leaf blades, sheaths, stalks, glumes, and kernels. In each of these the telia become readily evident from their dark brown to black color. They may occur in some part, usually the older portions, of the uredinial lesions; or they may develop quite independently. On the leaves and stalks, the telia commonly coalesce end to end, thus forming long, narrow, dark brown or black lines. On the glumes, these sori occur most commonly, although not exclusively, on the inner surface and are variously grouped. On the kernels, they may be external and evident, variously scattered or grouped, anywhere on the lobes; or they may be internal, that is, within the pericarp in spore pockets after a manner not unlike that noted for the uredinia (fig. 2).

FIG. 2.—Schematic drawing of a cross section of a rust-infected wheat kernel showing pockets or inclusions of rust spores within the pericarp

ON OTHER GRAINS AND GRASSES

The stripe-rust lesions on cereal and grass hosts other than wheat are in most respects similar to those on wheat. It has been observed, however, that there is considerable difference in the color of the uredinia on different grasses. Whether this is due to any actual difference in color of the urediniospores or is traceable solely to depth of spore mass has not been determined. Glume infection has been noted upon *Bromus marginatus* and *B. sitchensis*, but has not yet been observed on any other of the wild-grass hosts.

LIFE HISTORY AND MORPHOLOGY OF THE ORGANISM

Under congenial climatic conditions, the urediniospores of *Puccinia glumarum* retain their viability throughout the winter months and thus constitute an important factor in facilitating the natural spread of infection. Experiments conducted by Eriksson and Henning (*4*) led them to conclude that the germination of the urediniospores was dependent on the influence of low, even freezing temperatures. Particularly did this seem true of spores which had matured early in the season and therefore had been subjected to prolonged exposure to light and desiccation. One of the writers (Hungerford), however, has experienced no difficulty in securing prompt germination of these spores without first subjecting them to subnormal temperatures.

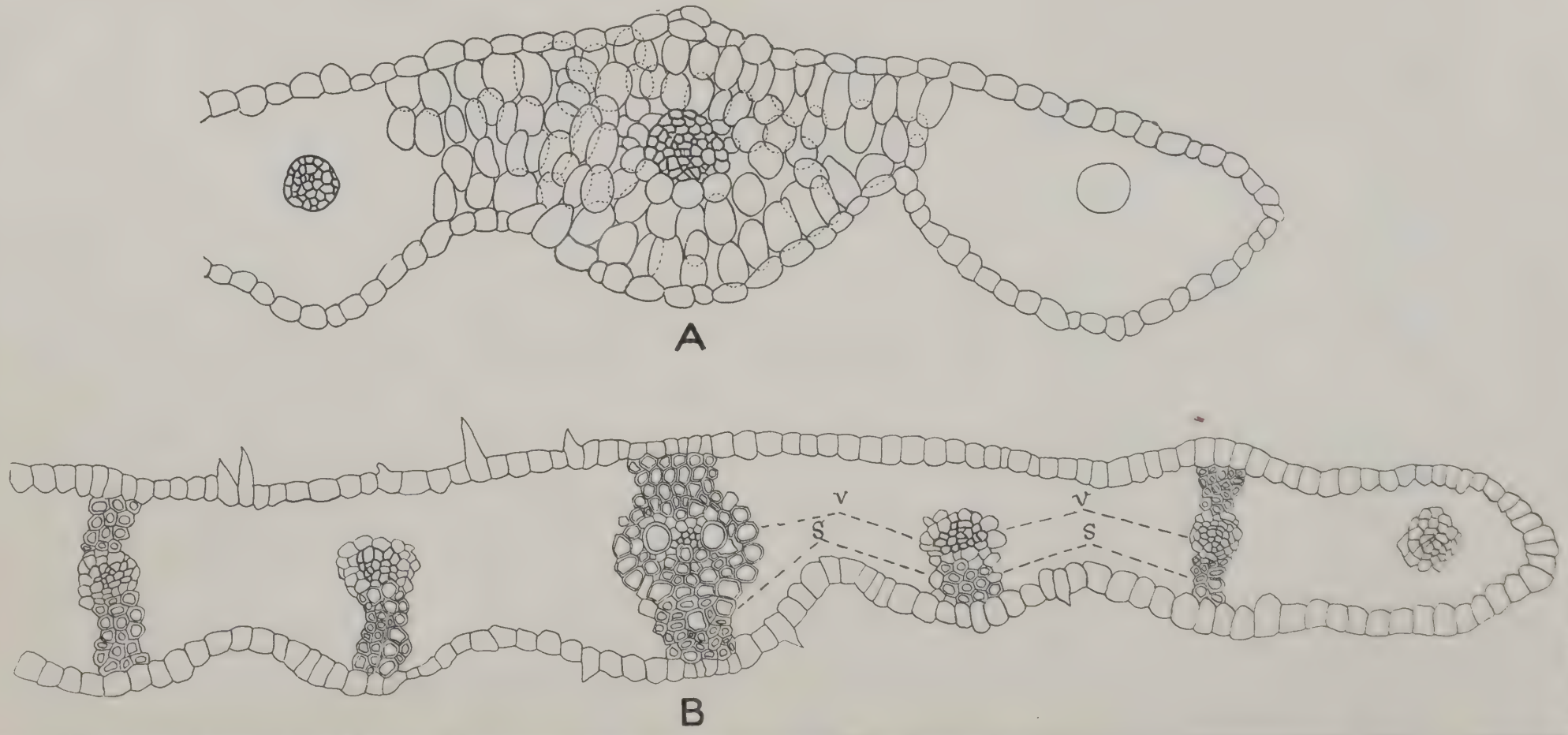


FIG. 3, A.—Schematic drawing of a cross section of a wheat-seedling leaf showing spongy mesophyll and vascular bundles, and absence of sclerenchymatous tissue. B.—Schematic drawing of a cross section of an adult wheat leaf showing vascular bundles, *v*, and supplementary sclerenchyma, *s*. These partitions divide the leaf into as many longitudinal segments of parenchyma

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According to Eriksson and Henning (*4*), the urediniospores of *Puccinia glumarum*, like those of *P. graminis*, produce a germ tube of fairly uniform diameter. It is almost invariably unbranched and contains throughout its length the yellow contents of the spore. Where germination takes place on the surface of a living host leaf, there develops at once an appressorium from which is derived the infecting thread or hypha which enters a stoma to proliferate later and form successive masses of mycelium or hymenia which give rise to uredinia and a new crop of urediniospores. Throughout the vegetative period of the host, the disease spreads from leaf to leaf and ultimately

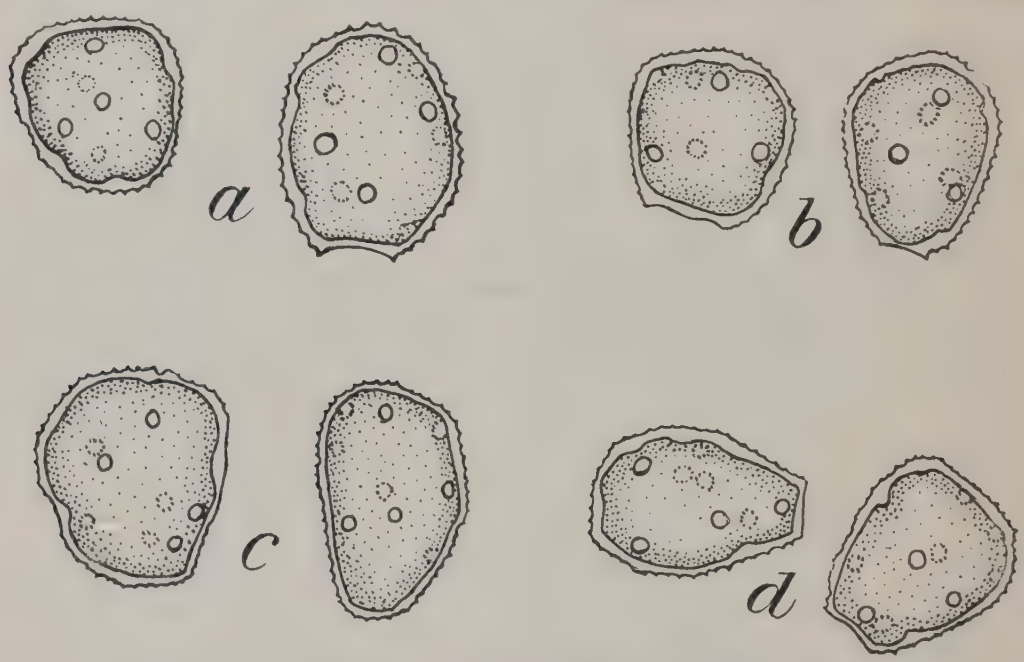

FIG. 4.—Camera lucida drawings of urediniospores of *Puccinia glumarum* on, *a*, *Triticum vulgare*; *b*, *T durum*; *c*, *Hordeum jubatum*; and *d*, *Elymus canadensis*. × 1000

to the entire plant. Under particularly favorable conditions in spring and early summer the amount of initial infection and subsequent development of stripe rust in any one season may be considerable and result in severe damage to the crop.

Marryat (*18*) states that infection resulting from *P. glumarum* obtains, as in the case of *P. graminis*, through entrance of the stomata by the germ tubes of the urediniospores.

URediniospores

The urediniospores of *Puccinia glumarum*, sometimes accompanied by hyphoid, incurved paraphyses, vary in form from globose to broadly ellipsoid. The colorless spore wall is moderately echinulate and varies in thickness from about 1 μ to 2 μ. The four to several germ-pores occur irregularly and, except under very favorable optical conditions, are seen with difficulty (fig. 4). According to Eriksson and Henning (*4*), the urediniospores measure from 16 to 19 μ by 25 to 30 μ in diameter. Grove (*6*), on the other hand, gives the dimensions as varying from 18 to 26 μ by 25 to 30 μ. As will be seen from Table I, the writers' figures, based on the measurement of 10,000 spores, show a variation of 12.7 to 25.9 μ by 15.7 to 33.8 μ. The average of 10,000 long and short diameters was 22.63 μ and 18.40 μ, respectively. The wide differences in the two diameters would seem to indicate the possible immaturity of some of the spores. Care was exercised in every instance, however, to confine the measurements to those which were fully mature. Subsequent to the preparation of the data presented in Table I, it was found that the size of the urediniospores apparently is governed to some extent by position on the infected leaf and consequent availability of nutriment. For example, 100 spores from uredinia near the base of a rusted leaf of Baart wheat averaged 18.90 μ by 25.64 μ; a similar number from the middle of the leaf averaged 18.71 μ by 24.84 μ while 100 spores from uredinia at the outer end of the leaf averaged 18.54 μ by 23. 12 μ.

TABLE I.—*Diameter measurements of unrediniospores of Puccinia glumarum*

No.	Host	Collection		Range of size in micra		Average of 200 spores	
		Place	Time	Short diameter	Long diameter	Short diameter	Long diameter
1	*Agropyron cristatum*	Moscow, Idaho	July, 1916	13. 5–24. 2	16. 3–27. 0	18. 33	21. 85
2	do	do	do	14. 5–25. 5	17. 5–29. 6	18. 63	21. 90
3	do	Hilmar, Wash	July 9, 1919	14. 1–23. 0	15. 8–29. 6	18. 07	22. 81
4	*Agropyron cristatum* (Importation from Russia).	Moscow, Idaho	July, 1916	14. 5–23. 9	18. 4–29. 3	19. 27	22. 25
5	*Bromus marginatus*	Pullman, Wash	June 14, 1915	14. 4–21. 8	16. 8–25. 8	18. 04	21. 43
6	*Bromus pacificus*	Friday Harbor, Wash.	June, 1916	15. 3–21. 3	18. 0–28. 3	18. 29	21. 72
7	*Bromus sitchensis*	N. Mt. Vernon, Wash.	do	14. 1–21. 2	15. 4–26. 7	17. 39	20. 66
8	*Elymus canadensis* [a]	Pullman, Wash	July, 1915	14. 7–23. 8	19. 7–33. 8	18. 81	24. 17
9	do	do	July 25, 1915	14. 6–21. 2	18. 1–31. 4	18. 05	23. 722
10	*Elymus condensatus*	Moro, Oreg	Sept. 10, 1916	14. 6–22. 8	18. 5–28. 6	18. 918	22. 817
11	*Elymus glaucus*	N. Mt. Vernon, Wash.	June, 1916	14. 9–24. 2	18. 4–30. 2	19. 02	23. 90
12	do	Friday Harbor, Wash.	do	16. 4–25. 9	16. 7–28. 6	18. 749	22. 071
13	do	do	do	14. 8–24. 1	17. 5–27. 7	17. 867	22. 268
14	*Hordeum jubatum*	Cherry Creek, Denver, Colo.	June 14, 1916	12. 7–21. 0	17. 4–28. 2	17. 294	22. 425
15	do	Denver, Colo	do	14. 2–23. 1	17. 2–27. 9	18. 36	22. 714
16	do [b]	Moro, Oreg	Sept., 1916	13. 7–20. 7	18. 0–27. 7	18. 42	23. 852
17	do	Garfield, Wash	do	14. 2–22. 0	18. 2–27. 9	18. 594	22. 553
18	do	N. Yakima, Wash	June, 1916	15. 3–23. 0	17. 2–30. 0	18. 780	22. 996
19	do	Selah, Wash	June 17, 1916	15. 3–23. 6	18. 6–31. 1	19. 323	23. 830
20	do [c]	Denver, Colo	June 14, 1917	15. 0–23. 8	17. 9 30. 2	20. 135	24. 648
21	*Hordeum murinum*	Los Angeles, Calif	May, 1916	15. 3–21. 7	16. 5–25. 6	18. 748	21. 732
22	do	Del Monte, Calif	do	14. 2–21. 7	16. 7–28. 5	17. 873	22. 537
23	do	Tehachapi, Calif	May, 1915	13. 7–21. 5	18. 4–27. 2	18. 999	22. 475
24	do	Campbell, Calif	May, 1916	15. 2–22. 6	18. 3–20. 3	18. 245	21. 448
25	*Hordeum nodosum*	Mt. Vernon, Wash	Sept. 2, 1916	14. 7–25. 1	17. 6–24. 9	18. 248	21. 128
26	*Hordeum vulgare*	Pullman, Wash	June 14, 1915	14. 4–21. 7	18. 0–29. 8	18. 973	22. 275
27	*Sitanion hystrix*	Redmond, Oreg	July 2, 1914	14. 5–22. 9	18. 0–27. 7	18. 431	22. 859
28	*Sitanion jubatum*	Ashland, Oreg	June 7, 1916	15. 3–23. 4	18. 7–30. 0	18. 462	23. 751
29	do	do	do	14. 2–21. 5	17. 7–29. 2	18. 554	23. 336
30	*Sitanion longifolium*	Red Mountain, Colo	Aug. 26, 1907	13. 7–25. 4	16. 4–29. 2	17. 556	21. 692
31	*Triticum compactum*	Pullman, Wash	Aug. 18, 1915	13. 8–22. 2	17. 5–29. 2	18. 126	21. 976
32	*Triticum dicoccum* (Buffum winter emmer).	Moro, Oreg	June 11, 1915	14. 0–21. 3	17. 7–28. 8	18. 067	22. 776
33	*Triticum durum*	Moscow, Idaho	June 15, 1915	14. 9–20. 4	17. 0–25. 4	17. 504	20. 803
34	*Triticum spelta*	do	July 2, 1919	14. 1–22. 6	16. 2–28. 5	18. 107	21. 303
35	*Triticum vulgare*	Bozeman, Mont	July, 1915	14. 7–23. 2	17. 9–32. 1	18. 245	22. 896
36	*Triticum vulgare* (Chul)	Corvallis, Oreg	June 10, 1915	15. 1–21. 9	19. 1–31. 1	18. 666	24. 172
37	do	do	June 12, 1915	15. 5–24. 6	18. 0–32. 1	19. 154	23. 287
38	*Triticum vulgare*	do	June, 1915	16. 0–23. 0	18. 0–27. 7	18. 721	23. 082
39	do [d]	Bozeman, Mont	July 26, 1915	15. 4–23. 6	19. 0–28. 4	19. 467	24. 181
40	do	Moscow, Idaho	June, 1915	14. 1–21. 3	16. 1–27. 8	17. 727	21. 077
41	do	Spokane Co., Wash	July 20, 1915	12. 7–23. 5	15. 9–27. 8	18. 517	22. 689
42	do	Brigham, Utah	June 23, 1915	14. 4–24. 2	15. 7–31. 1	18. 305	24. 319
43	do	Moscow, Idaho	June 15, 1916	15. 1–23. 3	17. 8–28. 0	18. 102	22. 038
44	do	Casa Blanca, Ariz	May 25, 1915	14. 0–20. 0	17. 9–27. 5	17. 947	22. 219
45	Wheat (species and variety not given).	Moro, Oreg	June 11, 1916	14. 0–23. 5	16. 8–29. 7	18. 322	23. 562
46	do	Freeman, Wash	June 19, 1916	14. 7–22. 4	17. 4–28. 4	18. 471	23. 039
47	Wheat [e]	Bozeman, Mont	Aug. 20, 1915	14. 8–23. 2	19. 0–30. 2	18. 888	24. 272
48	Wheat (Jones Winter Fife).	Pullman, Wash	June 22, 1916	14. 3–23. 3	17. 5–29. 5	17. 382	21. 636
49	Wheat	Eagle, Idaho	June 24, 1916	14. 5–22. 5	17. 8–27. 8	17. 911	22. 574
50	Wheat (mixed)	Mt. Vernon, Wash	June 14, 1916	13. 3–20. 6	17. 9–28. 0	18. 009	21. 705
	Grand average					18. 401	22. 628

[a] Spores very thick-walled, from 0.8 to 3.2 micra.
[b] Nearly all teliospores.
[c] Nearly all teliospores. Shows transition between unrediniospores and teliospores.
[d] Transition from unrediniospores to teliospores.
[e] Telial stage.

Levine (*16*) has shown that although resistant host plants and adverse environmental conditions always tend to dwarf the spores of *Puccinia graminis tritici*, regardless of the physiologic form, this reduction in size is not permanent. He has shown that when cultures from such spores are maintained under normal growth conditions the resultant spores are of characteristic size and shape. The same condition also doubtless holds for the spores of *P. glumarum*.

TELIOSPORES

The teliospores of *Puccinia glumarum* develop in hypophyllous or culmicolous sori. They occur in long, narrow lines; and in those wheat varieties where glume infection is common, form oblong blackish patches, in all cases covered by the epidermis. The spores, commonly 2-celled, are clavate to rounded or obliquely conical above, with smooth exospore which varies in thickness from about 1 μ to 3–6 μ for the thickened apex (fig. 5). They are brown, somewhat constricted, attenuate at the base, and measure, according to Eriksson and Henning (*4*), 16 to 24 μ wide by 30 to 40 μ long. In his description of the teliospores Grove (*6*) gives a width varying from 12 to 24 μ and a length of 30 to 70 μ. The writers' figures, derived from the measurement of thousands of mature teliospores, approach more nearly those of Grove, and vary from 12 to 27 μ by 30 to 65 μ. The spores, borne on pale, short and usually persistent pedicels, occur in groups surrounded by long sterile curved cells or paraphyses. According to Arthur (*1, p. 338–339*) the teliospores germinate at maturity.

Seed infection of wheat by *Puccinia glumarum*, which was first reported by Eriksson and Henning (*4*) and later by Blaringhem (*3*), has been noted by the writers most commonly in certain varieties, particularly Chul. The infection occurs in the pericarp of the wheat kernel, where it develops as mycelial pockets containing either urediniospores or teliospores or both (fig. 2).

This seed infection suggests potential importance from the standpoint of seed transmission, which problem is being investigated by one of the writers (Hungerford) and the results will be published separately.

THE FUNGUS IN RELATION TO DEVELOPMENT OF THE DISEASE

SEASONAL OCCURRENCE

As is true of other cereal rusts, stripe rust reacts to the conditions imposed upon it by temperature, relative humidity, soil, and other factors affecting the growth of both parasite and host. The absence of an alternate host and the relatively short period of viability of the urediniospores make more or less fortuitous the occurrence of general and destructive outbreaks of this rust. Given a source of inoculum of sufficiently wide distribution, accompanied by a combination of high relative humidity, relatively low fall temperatures, and complete susceptibility of host, a general and severe outbreak of seedling infection may develop during the fall. If the crop is sufficiently protected from alternate freezing and thawing during the winter to prevent winter killing, the mycelium of the rust fungus will hibernate and be present as a potential factor for the development of new urediniospores the following spring.

From the observational data in hand it is apparent that the further development and spread of stripe rust during the spring depend primarily on continuous high humidity and relatively low temperatures. On the other hand, it is true that a much more critical examination of meteorologic data and of other information bearing a possible relation to infection and spread of stripe rust in both the United States and Europe will need to be made before it can be stated very certainly what combination of conditions most definitely determines the seasonal occurrence and severity of stripe rust.

VIABILITY AND LONGEVITY OF SPORES

The literature extant on stripe rust contains but scant information on the duration of viability of the spores of *Puccinia glumarum*. Investigators, studying the problem of seasonal propagation of the rust, have emphasized the importance of hibernating mycelium, and have shown that even in the far northern countries of Europe, stripe rust may, and frequently does, survive the vicissitudes of winter as viable mycelium within the tissues of the host

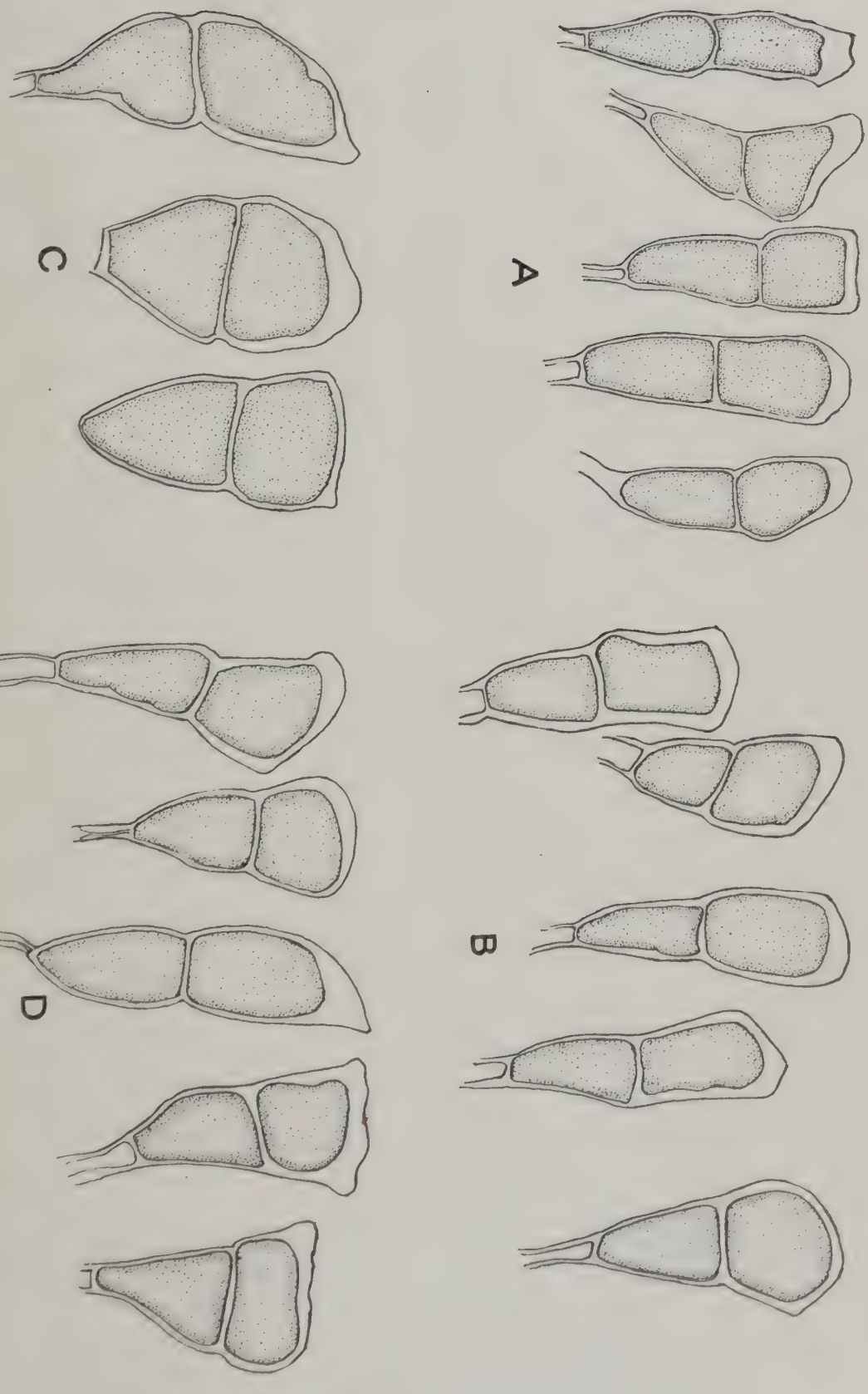

FIG. 5.—Camera lucida drawings of teliospores of *Puccinia glumarum* on, *A*, *Triticum compactum*; *B*, *Hordeum jubatum*; *C*, *Elymus striatus*; *D*, *Triticum vulgare*, showing variation in size and form of spores taken at random. × 1000

plant. Aside from the teliospore germination studies conducted by Eriksson and Henning (*4*) in the early 90's, but little further work has been done to determine either longevity or possible function of the teliospores of this rust. The investigation of this phase of the stripe-rust problem by one of the writers (Hungerford) is now in progress at Moscow, Idaho.

SOURCES OF NATURAL INFECTION

Until the two recognized races of *Puccinia glumarum* in North America have been critically studied in the light of their differential host relationships, knowledge of possible sources of natural infection will be but fragmentary and more or less uncertain. According to Hungerford (*11*), stripe rust already has been found to occur naturally in this country on wheat, barley, and rye, and 39 wild grasses. Two grasses very commonly infected are *Hordeum jubatum* and *Bromus marginatus;* and the form of the rust occurring on these hosts goes readily to wheat. *Hordeum jubatum* is common throughout the Northern States from Washington to Maine, yet the rust has not traveled eastward beyond 103° W. longitude. It probably is true, however, that the wild-grass hosts play an important rôle in the spread of stripe-rust inoculum in the form of urediniospores that have survived the winter and new urediniospores which have been produced by hibernated mycelium. Irrespective of any wild-grass hosts, there is always the possible winter survival of both urediniospores and mycelium in the infected tissues of the cultivated hosts, wherever winter cereals are grown. Volunteer wheat, barley, and rye, developing as they usually do very early after the first late summer rains, frequently become infected and serve as sources of inoculum for the infection of the fall-seeded crop.

Hungerford (*10*) conducted extensive studies to determine the possible relation of wheat kernels infected with *Puccinia graminis tritici* to the occurrence of initial infection in seedlings grown from such kernels. His evidence was wholly negative. The same author (*11*) obtained like results in his investigation of the problem of seed transmission of living mycelium and urediniospores of *P. glumarum*.

Eriksson and Henning (*4*) assert that it may be accepted as an established fact that this rust frequently lives from one crop season to the next in a dormant or resting-mycelium stage, present in infected seedlings of winter wheat. They do not show, however, that such winter survival of the fungus is necessary to its perpetuation, even in the far northern agricultural districts of Sweden. Indeed, their experimental study of the relation of such factors as humidity, light, and temperature to germination of the urediniospores would indicate that low temperatures enhance rather than inhibit their germination. They do not deny the fact that hibernating urediniospores, under favorable conditions, may assist materially in accelerating a severe epidemic of rust; but it is their opinion that the severity and extent of such an epidemic are more directly dependent on dormant mycelium.

Biffen (*2*) states that stripe rust is the most destructive of the wheat rusts common to England and that winter survival of *P. glumarum* under the conditions of England's winter climate is very largely due to viable urediniospores which have survived the winter.

Mehta (*19*), in his comparative study of the viability and germination of the spores of cereal rusts, found the urediniospores of *P. glumarum* to be short-lived and their vitality impaired by temperatures easily withstood by the urediniospores of *P. graminis* and *P. triticina*. His experiments indicated that the urediniospores of *P. glumarum*, exposed to temperatures as low as 2.5° to 5° C., retained their viability less tenaciously than do those of *P. triticina*. He found that 15 to 20 per cent of them survived an exposure of a month at the above temperatures.

The writers' observations on the probable overwintering of living mycelium agree with those of Eriksson and Henning. In connection with an experiment conducted by one of the writers (Hungerford), designed to determine the possibility of seed transmission of the disease, three lots of Chul wheat were sown in October, 1916. One lot of seed was badly infected with stripe rust. Another lot, similarly infected, had been given the modified hot-water treatment; and the third lot was of clean, rust-free seed. Somewhat later, through a misunderstanding, some plants of *Bromus marginatus* and *Hordeum jubatum* infected with *P. glumarum* were set out adjacent to the above plats of wheat. During the winter, both grasses died down to the ground while the wheat remained green. On April 25, 1917, the rust was found on three or four wheat plants immediately next to these grasses. Subsequently, the rust spread rapidly from this center of infection

and with the development of new leaves the grass plants also became heavily infected. From the foregoing, it would appear that viable urediniospores from one or both of the grasses had found lodgment on the leaves of the nearby wheat plants, and that the subsequent infection of the latter was traceable to spores produced in the grass hosts.

Rostrup (*20, p. 260*) is of the opinion that the stripe-rust fungus hibernates as living mycelium, and that on resumption of growth is followed promptly by the development of uredinia. Apparently the temperature permitting such renewed activity is relatively low, for, as pointed out by Henning and Bygdén (*9*), stripe rust not uncommonly appears shortly following the melting of the snow. In Denmark it was more or less prevalent in April of 1906, 1910, 1911, and 1914. In 1910 a new crop of urediniospores sufficiently mature to germinate was found as early as the first week of March.

Klebahn (*14*) tried to show by experiment the hibernating capacity of the urediniospores of *Puccinia glumarum*, but obtained only negative results. He was successful, however, in securing germination of urediniospores of *P. dispersa* Erikss. and Henn. that had survived the winter. Hecke (*7, 8*) studied the same problem in Austria and showed that the mycelium of *P. glumarum* could endure a temperature of −10° C., or lower. He further pointed out what is probably a fact, namely, that the mycelium of this rust organism will survive such winter conditions as may not prove too rigorous for the host.

K. Murashkinski, Director of the Western Siberian Laboratory of the Siberian Agricultural Academy, Omsk, Siberia, in correspondence with the senior writer, states that he had locally observed *Puccinia glumarum* on *Bromus unioloides* H. B. K. and *Elymus dahuricus* Turcz. in 1920 and 1921, and that infection seemed to be confined solely to these two grasses. Omsk is situated in latitude 55° N. and is subject to extremely low winter temperatures. The moderately heavy and continuous snow cover of that region from October until April, however, undoubtedly affords ample protection to both host and parasite, thus insuring the propagation of the rust organism by means of hibernating mycelium.

CLIMATE IN RELATION TO DEVELOPMENT OF STRIPE RUST

Unpublished meteorologic data furnished by D. E. Stephens,[4] for the period September, 1914, to September, 1915, inclusive, would seem to indicate beyond doubt the nice dependence of *Puccinia glumarum* on climatic conditions. During May and June, 1915, stripe rust developed at the substation to the point of a genuine epidemic. Given the necessary inoculum to insure a general distribution of seedling infection during September and October, 1914, the heavy and almost continuous snow cover of the winter of 1914–15 furnished ideal overwintering conditions. Reference to Table II will show how favorable for infection were the precipitation and temperature conditions of September and October, 1914. It will be noted also that these same factors during the spring and early summer months of 1915 were such as to promote the further development and spread of the rust. If these data are compared with those for September and October, 1915, and those of the spring and early summer months of 1916, it will be noted that the factors favoring early emergence and normal subsequent fall growth of volunteer wheat were quite as ideal during September and October, 1915, as they were during the same period in 1914. Moreover, assuming that the 1915 epidemic had its origin in hibernating mycelium arising from infection during the autumn of 1914, the amount of available rust inoculum (urediniospores) must have been very much more abundant than in the fall of 1914. It also should be noted that the winter of 1915–16 was characterized by a nearly continuous snow cover. The temperature and moisture conditions of March, April, and May, 1916, were not inimical to the development of stripe rust, yet 1916 was not a rust year. Commercial wheat fields inspected in June showed but a trace and only the more susceptible varieties growing on the station farm were infected.

[4] STEPHENS, D. E. REPORT OF THE SHERMAN COUNTY BRANCH STATION, MORO, OREGON, 1914–16. [Unpublished. Copies in Office of Cereal Investigations, U. S. Dept. Agr., Washington, D. C.] (C. E. HILL, joint author, 1915–16.)

TABLE II.—*Meteorologic data for the period January to December, 1914, 1915, and 1916, inclusive, recorded at the Sherman County Branch Station, Moro, Oreg.*

Month	Air temperatures (°F.)									Precipitation (inches)			Evaporation (inches)		
	Maximum			Minimum			Mean								
	1914	1915	1916	1914	1915	1916	1914	1915	1916	1914	1915	1916	1914	1915	1916
January	43	41	20	30	10	10	36	26.0	15.0	2.20	1.75	1.10	------	------	------
February	39	52	35	27	23	26	33	36.0	30.5	1.16	2.31	2.43	------	------	------
March	54	70	50	34	30	35	44	45.0	42.5	.11	1.27	2.05	------	------	------
April	59	75	59	39	32	37	49	50.8	48.0	2.06	.65	.75	4.01	5.23	4.80
May	70	78	61	42	34	39	56	53.0	50.0	.76	2.06	1.37	7.43	5.73	5.68
June	72	93	72	47	40	47	59	60.0	59.5	.66	.36	1.98	8.29	8.24	6.80
July	85	96	74	56	40	51	70	64.8	62.5	.08	.57	.92	11.43	8.95	7.88
August	85	100	82	54	46	54	69	71.3	68.0	Trace	.05	.15	9.64	9.61	7.50
September	67	86	72	44	36	45	55	57.3	58.5	1.05	1.14	.33	4.40	5.23	4.68
October	59	73	62	40	33	34	49	50.6	48.0	1.48	.23	.39	2.19	2.93	2.81
November	47	54	41	30	25	26	38	38.2	33.5	.88	2.89	1.69	------	.45	------
December	28	53	35	17	11	23	23	32.8	29.0	.88	1.61	1.32	------	------	------

According to Lang (*15*), who has made a careful investigation of climatic factors in relation to the occurrence of stripe rust in Europe, the following comprise the conditions typical of a severe epidemic: An abundance of hibernating mycelium, followed by the development of uredinia in late March or early April. This first crop of urediniospores, favored by congenial conditions for germination and incubation, will give rise to a second, and from the second a third crop of spores. Thus, by early May, assuming conditions to be ideal, a severe and general epidemic will have developed. Spring wheat sown in April will become infected by the middle of May, and by the end of the month the second principal outbreak of the rust will have occurred.

Litvinov (*17*), commenting on the probable causes which contributed to the severe epidemic of stripe rust in central Russia in the summer of 1914, attributes the attack to favorable climatic factors. The spring of 1914 was relatively cool, but free from recurrent thaws. The general coolness of the spring contributed to the early development of urediniospores as contrasted with *Puccinia triticina*. His observations were confined to experiments with varieties of spring wheat, in which he noted that susceptible early varieties were much more subject to damaging infection than were late-sown varieties of approximately equal susceptibility. Jenkin and Sampson (*13*) in Wales likewise noted a correlation between date of seeding and severity of infection, but their results indicate that the severest infection develops on the latest-sown rather than on the earliest-sown plats. It is possible that this difference in correlation may be traced to differences in contingent climatic conditions which characterized the spring of 1914 at Voronezh and that of 1920 at Aberystwyth.

Although the foregoing instances of severe, even damaging, infection in spring wheat would seem to controvert the evidence adduced by the majority of the students of stripe rust to the effect that serious infection seldom develops in spring wheats, it should be pointed out that even such instances may be concerned with epidemics originating from mycelium which may have hibernated in some near-by grass host or in volunteer wheat plants.

Thus it is conceivable that climatic conditions which favor a general seedling infection during the autumn months may contribute to the development of a high potential for a subsequent and destructive epidemic during the spring and summer. But it is apparent that unless the fungus is favored by optimum spring conditions it will not develop to economic proportions.

It is obvious from the foregoing that until a more extended and critical study has been made of all possible factors influencing infection by, and subsequent development of, the stripe-rust fungus, it will not be possible to answer the many perplexing questions that must arise in the mind of the investigator.

CONTROL OF STRIPE RUST

The adoption of varieties of wheat resistant to stripe rust is the most practical means of control. Jenkin and Sampson (*13*) found that of the more than 90 varieties and strains of wheat

included in their experimental study of varietal resistance to both *Puccinia glumarum* and *P. graminis*, Garton's Early Cone and Percival's Blue Cone proved resistant to both rusts. They found that certain varieties, seemingly rust-resistant, were merely rust-escaping because of their more rapid growth and maturity. The general adoption of such spring wheats undoubtedly would serve to reduce the danger of loss from stripe rust, but it is hardly probable that this would apply to winter varieties for the reason that the occasional presence of hibernating mycelium would make possible early and destructive outbreaks of stripe rust.

As in Europe, so also in the United States, wheat varieties differ markedly in their susceptibility to stripe rust. Moreover, varieties differ in susceptibility to infection both in the heads and in the leaves. Observations made by writers in 1915 at Corvallis, Oreg., on Chul wheat showed this variety heavily infected in the floral parts, especially the glumes, while but slight infection was noted on the blades and sheaths. Kernels from these infected heads were later examined microscopically and found to contain abundant internal infections. A number of other wheat varieties growing alongside showed no infection on the floral parts but a considerable amount of it on the leaves. One of the writers (Hungerford) has made similar observations. An excerpt from a summary of notes taken by him on an inspection trip made in 1916 presents the following interesting data on the subject of varietal resistance to stripe rust: "There seemed to be not only a vast amount of difference in the susceptibility of the various varieties of wheat to stripe rust but also there was a difference in the amount of infection appearing upon the glumes and on the kernels. Chul wheat did not seem to be especially heavily infected on the leaves, but was, in every case where noted, infected in the glumes to a marked degree."

During the first year (1915) stripe rust was observed in the United States, marked varietal differences in susceptibility to leaf infection were noted.[5] In a series of wheat varieties growing in adjacent twentieth-acre plats at Moro, Oreg., the leaves of Dale (C. I. No. 4155) were the most heavily infected, averaging about 85 per cent infection. Hybrid 123 (C. I. No. 4511), Baart (C. I. No. 1697), Little Club (C. I. No. 4066), Purplestraw (C. I. No. 1915), Turkey selection (C. I. No. 2998–1), and Beloglina (C. I. No. 2239) also were rather heavily infected, the infection ranging from about 75 per cent to 40 per cent in the order given. The other wheat varieties in the series all showed infections varying from about 30 per cent to a mere trace. During the same year (1915) Little Club showed about 65 per cent of infection at Moscow, Idaho, while other varieties there showed only slight infections or none at all. Subsequent observations and experiments by Hungerford and Owens (*12*) have shown more fully the relative susceptibility of different wheat varieties to stripe rust.

SUMMARY

(1) Collections of *Puccinia glumarum* were made in North America by various American botanists during the 90's and the early years of the present century. These collections were distributed under names other than *P. glumarum*.

(2) *Puccinia glumarum* was found and recognized in the United States by the late F. Kølpin Ravn of Copenhagen, Denmark, in May, 1915.

(3) The common name, stripe rust, is proposed in lieu of yellow rust or golden rust as a common name for the disease caused by *Puccinia glumarum*. This accords with the Italian name, *ruggine striata del grano*.

(4) *Puccinia glumarum* is now known to occur from British Columbia to Mexico and eastward to 103° W. longitude. It has been found in all of the Pacific and Intermountain States except Nevada and New Mexico.

(5) Stripe rust is a disease of considerable economic importance in Great Britain, Northern and Central Europe, North Africa, Japan, and India. Its destructiveness in the United States would undoubtedly be fully as serious as that experienced in "rust years" in Europe should it become established in the soft red winter-wheat areas east of the Mississippi River.

(6) *Puccinia glumarum* is now known to occur in nature on 34 wild grasses common to the United States in addition to the cultivated hosts, wheat, barley, rye, spelt, and emmer.

(7) Certain varieties of wheat apparently are much more subject to glume and kernel infection than others. All susceptible varieties show general leaf infection.

(8) An aecial host for *Puccinia glumarum* has not yet been discovered.

(9) The dimensions, particularly the length, of urediniospores produced on the leaves vary according to location on the leaf. Those occurring in ure-

[5] These early observation were made jointly by two of the writers (Humphrey and Johnson) and Dr. F. Kølpin Ravn.

dinia nearest the base of a given leaf are larger than those from uredinia located near the distal end.

(10) Infection of most hosts and varieties is confined to the leaves and culms.

(11) The uredinia and telia of *Puccinia glumarum* not infrequently occur on the kernels and glumes of certain varieties of wheat. Such infection usually causes noticeable shrinkage of the kernels and apparently affects their viability.

(12) The severity and spread of stripe rust in any given locality devoted to winter wheat depend primarily on (*a*) fall weather conditions favorable to seedling infection; (*b*) successful hibernation of mycelium; (*c*) spring weather conditions favorable to germination of urediniospores.

(13) *Puccinia glumarum* in the Pacific Coast States has been found capable of winter survival by means of living mycelium or viable urediniospores.

(14) Varietal-resistance studies indicate the existence of several varieties of wheat which are highly resistant to *Puccinia glumarum*. Among the most resistant are Turkey (C. I. No. 1558), Turkey (C. I. No. 1750), Alton (C. I. No. 1438), Prohibition (C. I. No. 4068), and Red Russian (C. I. No. 4222).

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A STUDY OF BACTERIAL PUSTULE OF SOYBEAN, AND A COMPARISON OF BACT. PHASEOLI SOJENSE HEDGES WITH BACT. PHASEOLI EFS.[1]

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INTRODUCTION

In the fall of 1916 badly spotted soybean leaves were received from Texas. The leaves were thickly covered with small, brown, angular, slightly raised spots (Pl. 2 A), from which a yellow organism resembling *Bacterium phaseoli* EFS. was isolated, and inoculations with this produced the disease on healthy soybean plants. The same disease was observed by Erwin F. Smith in 1902 on leaves from Charleston, S. C., and by the writer in the Dismal Swamp, Virginia, in 1917. In both cases an organism like *Bacterium phaseoli* was isolated. In later years it has been received from other localities.

HISTORY OF THE DISEASE

The first mention in literature of this disease is a brief note by Smith(*17*)[2] in 1904; and the leafspot referred to by the same author in "Bacteria in Relation to Plant Diseases" (*18, v. 1, p. 92; v. 2, p. 69*) is the one here under discussion (oral communication), but no description of the disease or organism was given. Heald (*6, p. 41, 71*) in 1905 and again in 1906 (*7*) described very briefly a bacterial blight of soybean, but no cultural studies were made and it is impossible to tell with certainty whether the disease which came under his observation was the one discussed here or the leafspot described by Johnson and Coerper (*10, 4*), resembling the pustule disease in certain stages but caused by a white organism, *Bact. glycineum* Coerper, or whether it was the one attributed by Wolf (*22*) and Shunk (*14*) to *Bact. sojae* Wolf, which resembles *Bact. glycineum* so closely that it is questionable whether one or two diseases are involved.

Clinton (*3, p. 444–445*) in 1915 gave a more detailed description of a bacterial leafspot of soybean and considered it identical with the bacterial disease of wax and Lima beans and due to *Bact. phaseoli* EFS., but made no cultural studies. Bryce (*2*) in 1917 reported the occurrence in Canada of a bacterial blight of soybean leaves, but accompanied this statement with no description; and the Plant Disease Survey of the United States Department of Agriculture (*5, p. 116*) recorded a bacterial leafspot on soybean in Pennsylvania and Indiana in 1918, with no clue as to which bacterial disease was meant

Three other species of bacteria are known to be infectious to soybean in the United States: *Bacillus lathyri* Manns and Taubenhaus (*11, p. 12; 21, p. 141*), *Bact. solanacearum* EFS. (*19*), and *Bact. flaccumfaciens* Hedges(*8*), but there is no danger of confusing any of the diseases caused by these organisms with the one under discussion.

In 1902, Smith isolated from soybean leaves the yellow bacterium resembling *Bact. phaseoli* EFS., and reproduced the disease on healthy plants by pure culture inoculations. Infections resembling those caused by *Bact. phaseoli* EFS. were also produced on varieties of *Phaseolus* sp. But, although several attempts were made, no infection with very virulent colonies of *Bact. phaseoli* EFS. isolated from Phaseolus was ever obtained on soybean by Smith (oral communication). Thus the question as to the identity of the two parasites has long been an open one, and in an attempt to solve this problem the writer undertook the present work, a preliminary account of which has been published (*9*).

GEOGRAPHICAL DISTRIBUTION

Little is known in regard to the geographical distribution of this disease. The writer has received material from Texas (1916), Virginia (1917 and

[1] Received for publication Apr. 19, 1924—issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 250-252.

1921), Arkansas (diseased seed, 1919), Louisiana (1922), Kansas (1923, 1924), and Delaware (1924) only, but as Smith obtained it in 1902 from South Carolina, it is quite probable that some, at least, of the "bacterial leafspot" of soybean reported from time to time by various investigators has been due to the organism herein described.

This organism or *Bact. glycineum* was present in South Dakota in 1922, but the material sent in was very dry and attempts at isolation of the parasite failed.

SIGNS OF THE DISEASE

The disease is characterized by angular reddish-brown spots on the leaves, varying in size from minute inconspicuous specks to large irregular brown areas involving a considerable portion of the leaf (Pl. 1, A to G). The latter are caused by a fusion of the smaller spots and are not uniform in color, but mottled in appearance. The original small spots can usually be distinguished in these larger brown areas caused by their fusion. Portions of the large spots frequently drop out, leaving the leaf ragged. The browning sometimes follows the veins (Pl. 2, B and C).

A common and conspicuous, though not universal, sign of the disease is a pronounced yellowing. When present, it occurs as a yellow border around each individual spot or as a yellowed area thickly beset with tiny brown spots or inclosing larger brown areas formed by the expansion and fusion of the same (Pl. 1, A to G). This yellow color varies from a pale yellowish green to a light cadmium (*13*).

The first signs of the disease are very small, inconspicuous, pale green or sometimes reddish-brown spots (Pl. 1, D), usually slightly but distinctly elevated in the center. Sometimes the whole spot is elevated, making a minute pustule (Pl. 1, F), sometimes only the central portion, which may be lighter colored than the pale yellowish-green periphery or may be reddish brown. These intumescences may be confined to either surface of the leaf or may extend through it and appear on both sides. They finally rupture the epidermis but rarely if ever attain a size of more than 1 mm. in diameter, and as the spot grows older they usually collapse, shrivel, or slough off. They are truly tumorlike to this extent, that they are due to both hypertrophy and hyperplasia (Pl. 3, A, C, D). *At no stage is there a water-soaked appearance of the tissues such as is so striking in the early stages of the blight caused by Bact. glycineum Coerper.*

In August, 1923, at the experimental farm of the University of Wisconsin the writer observed on many varieties of soybeans a heavy infection of *Bact. glycineum* and confirmed a previous belief that when young infections are present the two diseases are easily distinguishable, but at a later stage only the isolation of the parasite can insure a correct diagnosis.

The writer has observed infection of the pods only once, viz, in some greenhouse experiments on the Hahto soybean, the pods of which are less hairy than those of the other varieties used up to that time; but in all probability it occurs frequently under favorable field conditions. The spots were brown and slightly raised and the organism was isolated therefrom.

MICROSCOPIC APPEARANCE

Microtome sections through the pustules on Hahto soybeans three weeks after inoculation show that the bacteria (stained with carbol fuchsin)

EXPLANATORY LEGEND FOR PLATE 1

Natural infections and greenhouse inoculations. All natural size except F. Paintings by James F. Brewer.

A to I, *Bact. phaseoli sojense* Hedges:

A.—Natural infection on Mammoth Yellow soybean. Collected at Arlington, Va., October 7, 1921.

B.—Natural infection on Tarheel Black soybean. Collected at Arlington, Va., October 7, 1921.

C.—Natural infection on Barchet soybean. Collected at Arlington, Va., October 7, 1921.

D.—Natural infection on Mammoth Yellow soybean. Very early stage which might easily be overlooked. Collected at Arlington, Va., October 7, 1921.

E.—Artificial infection on Hahto soybean, inoculated March 11, 1919. Painted April 3, 1919; 3 weeks old. Pustule stage.

F.—Detail from Figure E showing pustule. ×10 ca.

G.—Artificial infection on Wilson Black soybean, inoculated March 12, 1920. Painted May 28, 1920; 6 weeks old.

H.—Bush string bean leaf sprayed with colony 6, October 20, 1919 (through bush string bean) January 19, 1920. Painted February 9, 1920; 3 weeks old.

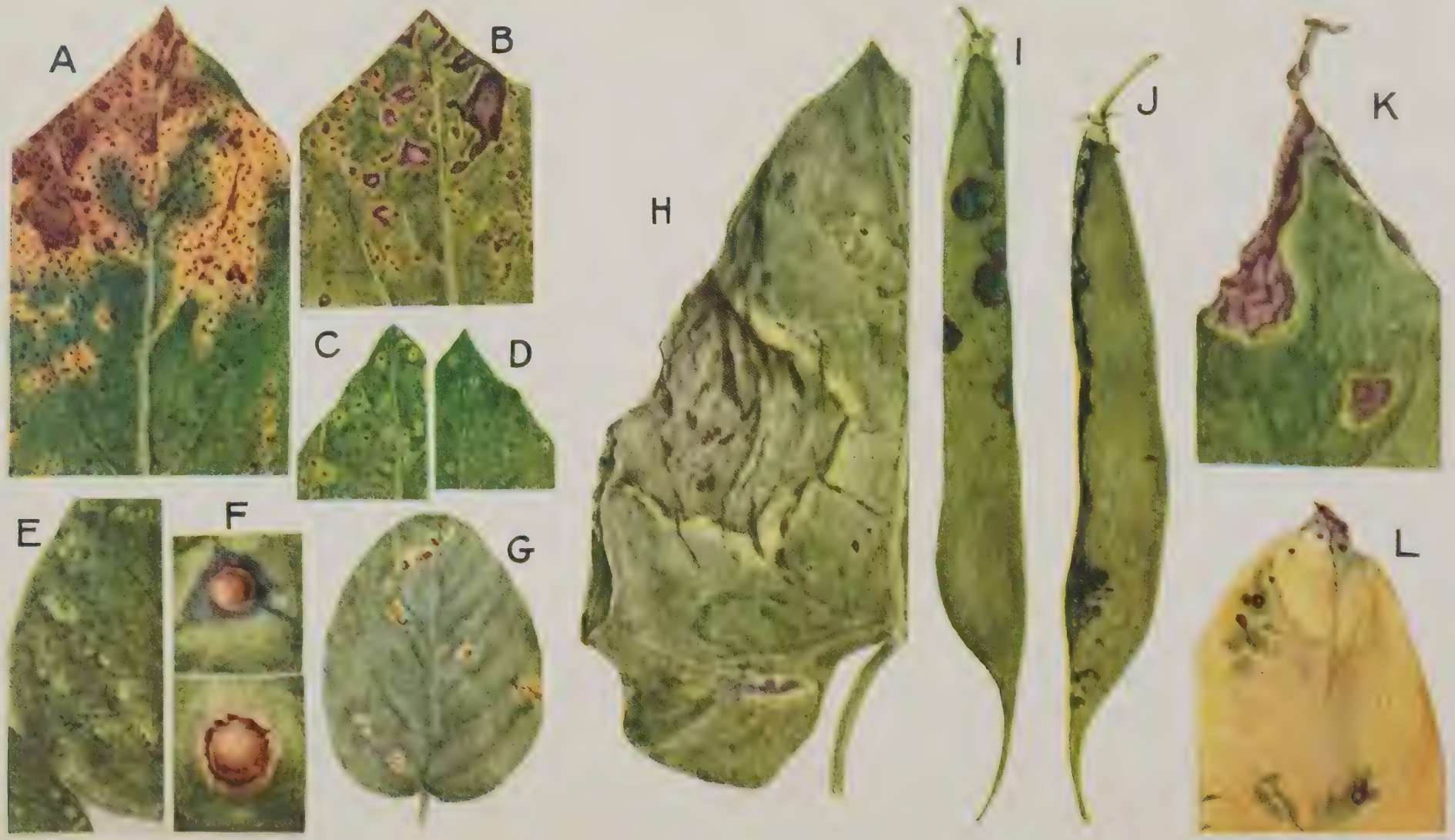
I.—Bush string bean pod; inoculated March 1, 1920, with colony 6, October 20, 1919 (through bush string bean). Painted March 22, 1920; 3 weeks old.

J to L, *Bacterium phaseoli* EFS.:

J.—Secondary infection on bush string bean pod on plant inoculated January 2, 1920 (in blossom at the time). Painted February 26, 1920.

K.—Bush string bean leaf, inoculated January 2, 1920. Painted February 7, 1919, 5 weeks old.

L.—Bush string bean leaf, inoculated January 2, 1920. Painted February 7, 1919, 5 weeks old; showing only islands of green.


A
B
C
D
E
F
G
H
I
J
K
L

are present in abundance, and that the swelling is caused chiefly by hypertrophy and hyperplasia of the parenchyma (Pl. 3, A, C). In some cases there was also striking hypertrophy of the epidermis cells (Pl. 3, D), but no hyperplasia was observed in this layer. The bacteria occur throughout the intumescence and there are bacterial cavities occupied by masses of bacteria, also innumerable areas where individual rods can be clearly seen (Pl. 3, B).

The tissues of a pustule were tested with KI and H_2SO_4 for cork seven weeks after inoculation. The cell walls gave the yellowish-brown reaction indicating suberized tissue, although none of the cells resembled cork cells in appearance. In fact, only a very small portion of the cell walls in the diseased area, whether in the swollen portion or not, showed the blue cellulose reaction.

ISOLATION OF THE PARASITE

The parasite is easily isolated from fresh material by means of agar poured plates. In some cases there was no previous sterilization of the leaf surface, but in the majority of cases $HgCl_2$ 1:1000 was used, the leaf being dipped first in alcohol, and subsequently thoroughly rinsed in sterile water to remove the $HgCl_2$. It was found that 10 to 20 seconds was a good average time for sterilization. The tissue was then crushed in a tube of sterile water or beef bouillon with a sterile rod and diffusion was allowed to take place for 10 to 20 minutes, or longer if the tissues were dry or microscopical examination had shown comparatively few bacteria present. The writer has safely used distilled water for rinsing the leaves and preparation of the dilutions, although this procedure has caused trouble with certain more sensitive organisms. The colonies are usually visible to the naked eye in 2 to 3 days but occasionally fail to appear until 4 or 5 days have passed.

INOCULATIONS ON SOYBEAN WITH BACTERIUM PHASEOLI SOJENSE

The disease has been reproduced repeatedly on sound soybean plants in the hothouse both by spraying and by rubbing with absorbent cotton wet in a water suspension of a culture of *Bact. phaseoli sojense* (Pl. 1, E to G; Pl. 2, B to D). The best results were obtained when young rapidly growing plants were used and were kept moist for at least 36 hours after inoculation. This was done by placing them in inoculating cages, the interior of which was thoroughly wet previous to inoculation. The cages were covered with heavy Manila paper and the plants were kept moist by repeated sprayings with bacterial suspensions during the 36 to 72 hours.[3] If very small plants were used and the only available inoculation cage was large, pans of water or large plants with plenty of transpiring surface were added.

Good infections were also obtained when the plants were covered with paraffined bags, but these did not hold the moisture as well as the cages and it was more difficult to respray the plants. Placing the sprayed or rubbed plants under a bench in the shade was sometimes sufficient.

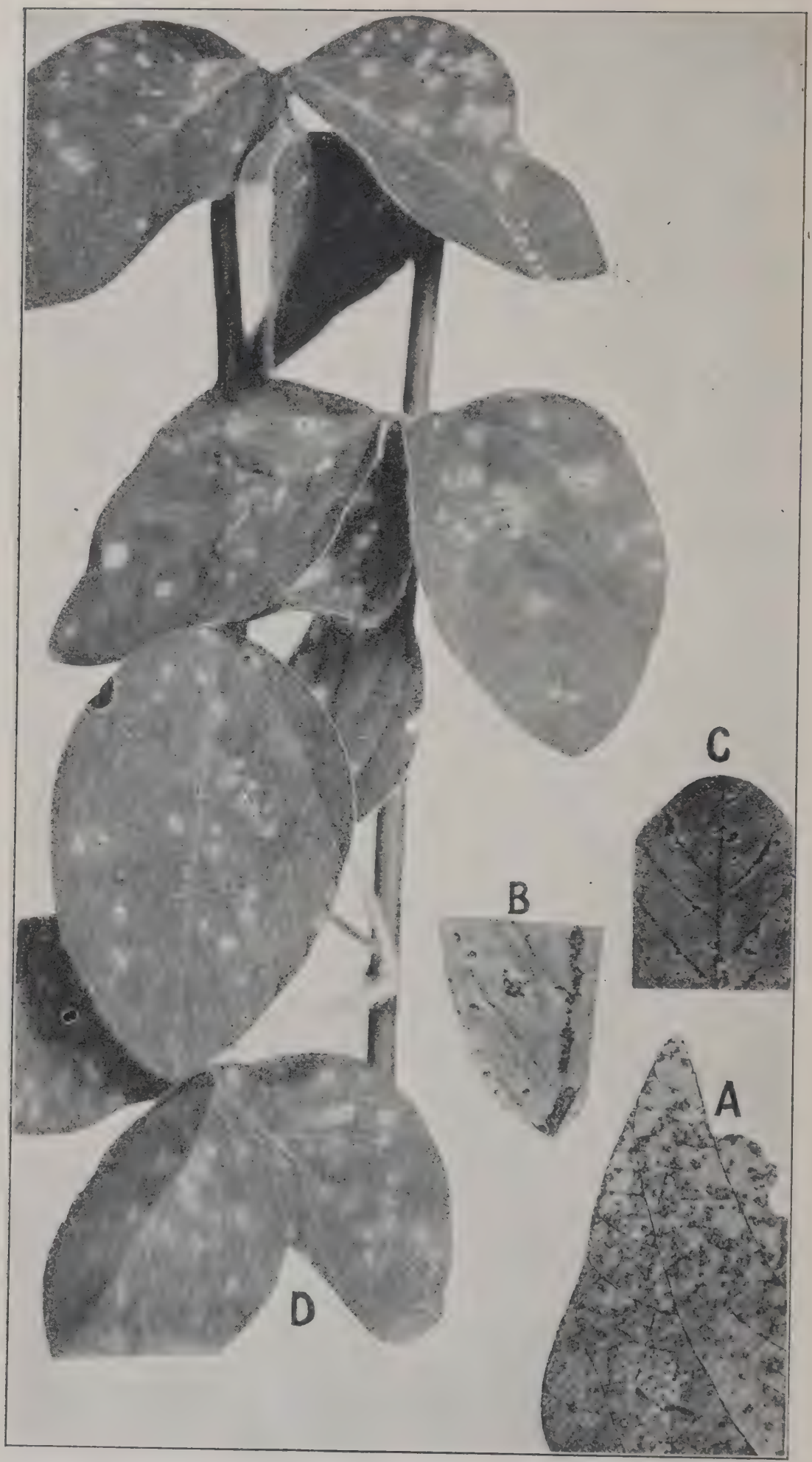
The first signs of infection usually appeared in 5 to 9 days, although in some cases there was a longer incubation period. Generally, the pustules were plainly visible in less than 10 days and the infection progressed as described under "Signs of the disease," above. Under greenhouse conditions the leaves rarely were as badly diseased as in the field. This was probably due to the fact that the soybean plants themselves did not thrive particularly well in the conditions prevailing in the Washington hothouses.

SUSCEPTIBLE VARIETIES OF SOYBEANS

Successful inoculations were made upon the following varieties of soybeans: Ito San, Medium Yellow, Mammoth Yellow, Hahto, Wilson Black, Hollybrook Early. No infection was obtained on Wilson Fine, although plants of Ito San and Medium Yellow of the same age (3 weeks) inoculated at the same time with the same culture became badly diseased.

Natural infection was observed in the fall of 1921 on the following varieties at Arlington Farm, Va.: Auburn, Austin, Barchet (Pl. 1, C), Biloxi, Buckshot, Chiquita, Edna, Fairchild, Flat King, Hahto, Hinangdon, Hollybrook, Honkong, Hoosier, Laredo, Mammoth Brown, Mammoth Yellow (Pl. 1, A and D), Manchuria, Mandarin, Midwest, Mikado, Mixture, Tarheel Black (Pl. 1, B), Tokio, Wisconsin Black, S. P. I. 49832,[4] 49918, 51042, 49834, 51043 (new introductions by S. P. I. with no varietal names), S. P. I.

[3] The plants were never kept in the cages as long as 72 hours when the temperature was high.
[4] Office of Seed and Plant Introduction, U. S. Dept. of Agriculture.



Bacterial Pustule of Soybean

Plate 2

Bacterium phaseoli sojense on soybean. (Photographs by JAMES F. BREWER)

A.—Natural infection, Texas. ×1
B.—Artificial infection 6 weeks old, with colony through Lima bean. ×1½
C.—Artificial infection 7 weeks old, with colony directly from soybean. ×1
D.—Soybean 4 weeks after spraying with colony passed through Lima bean. ×1½

49920, 50440, 50441, 51045, 51046, 52345, 52346, 52347, 52348, 52349 (Chinese varieties introduced in 1920–21).

Midwest was a particularly susceptible variety. There were few, if any, sound leaves. Hollybrook, Mikado, Tokio, Mammoth Brown, Hahto, Tarheel Black (Pl. 1, B) and some of the Mammoth Yellows (Pl. 1, A) were also badly infected. Buckshot was so slightly diseased that some search was required to find infected leaves. Mandarin and Biloxi also had a very small percentage of infection.

INOCULATIONS ON PHASEOLUS WITH BACTERIUM PHASEOLI SOJENSE

In an effort to determine whether the soybean organism was identical with *Bact. phaseoli* EFS., many cross inoculations were made. All inoculation experiments were carried on in the greenhouse. Infections were obtained on the leaves and pods of a number of the members of the genus Phaseolus with the soybean organism (Pl. 1, H and I; Pl. 4, A to C, E and F). On the other hand, repeated attempts to infect the soybean with *Bact. phaseoli* EFS. met with failure until inoculation was made under very abnormal conditions described later.

Successful inoculations were made with the soybean organism on Climbing Lima, King of the Garden Lima, bush string bean, dwarf bush string bean, dwarf bountiful string bean and dwarf wax bean.

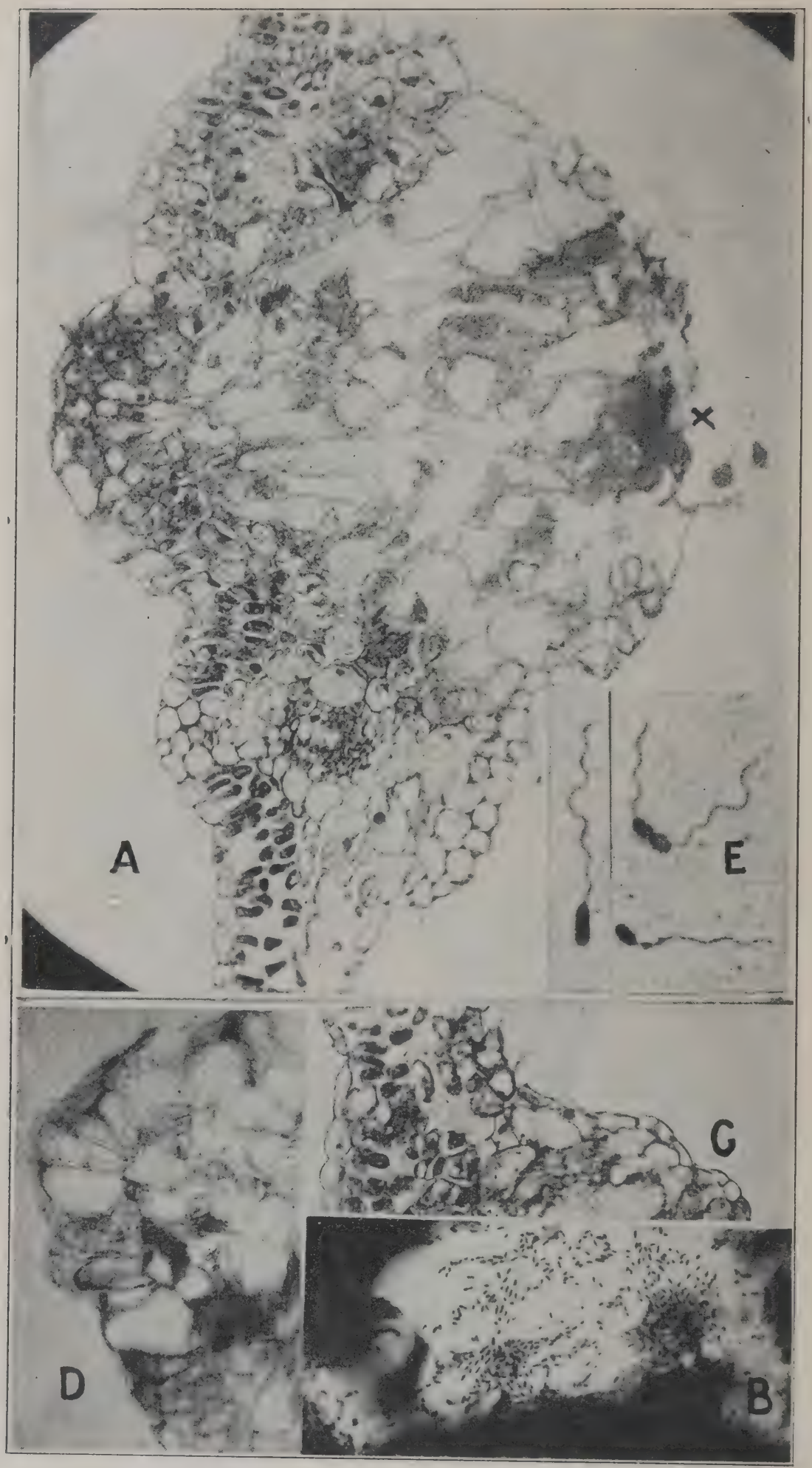
INOCULATIONS ON LIMA BEAN

On Lima bean leaves the soybean organism produced a reaction unlike that on soybean. No pustules were formed and sections through a spot 17 days after inoculation showed neither hypertrophy nor hyperplasia. Bacterial cavities and masses of bacteria were present. In only one case out of many was there observed even a slight elevation of the epidermis. Furthermore, if the infection made any progress after the appearance of the first small red spots or pale green ones with red centers, the spots became water-soaked and could not be distinguished from those caused by *Bact. phaseoli* EFS. In the best infections there was also yellowing in the later stages and a distortion of the very young leaves. In general, *Bact. phaseoli sojense* infected Lima bean leaves much more slowly than did the organism described from Phaseolus (Pl. 4, C, D, and H) except when the former's virulence for Lima was apparently increased by previous passage through Lima or bush string bean. The tiny spots sometimes did not appear for 2 weeks or more and then were very scattering and not infrequently failed to increase in size (Pl. 4, C). The plants were inoculated in the greenhouse by spraying and were kept moist in inoculation cages 48 to 72 hours, as a hot moist atmosphere is very favorable to infection of Phaseolus with both *Bact. phaseoli* EFS. and *Bact. phaseoli sojense.*

The first good infections on Lima beans were obtained on the green pods by spraying them July 13, 1917, with a water suspension of a 2-day-old potato culture of the soybean organism and keeping them wrapped in oiled paper 7 days. One side only had been sprayed, and when the paper was removed this side was covered with tiny water-soaked spots while the other was perfectly free. Three days later these water-soaked spots were very marked and there were still none on the unsprayed side. Bacterial ooze was also present. Later, the spots turned red but they enlarged little, if any, after the first 10 days (Pl. 4, E, F). The parasite was reisolated, and one of the resulting colonies produced excellent infections on both soybean leaves and pods, and on Lima bean pods, which were treated as described above. The Lima bean leaves were not inoculated.

INOCULATIONS ON DWARF BUSH STRING BEANS

Dwarf bush string bean was much more susceptible than Lima (with the exception of the latter's pods) to *Bact. phaseoli sojense* previous to its passage through a species of Phaseolus. The young leaves of this variety inoculated in the greenhouse September 30, 1919, were thickly covered with water-soaked spots on the ninth day after inoculation (no records made earlier). Two days later the center of the spot was becoming discolored but there was never much, if any, enlargement of it thereafter, nor was there any yellowing (Pl. 4, A). The spots were full of bacteria and the parasite was reisolated from them. These inoculations were made on plants 2 weeks old in an inoculating cage in which they were kept moist for 72 hours. There was very slight infection on King of the Garden Lima plants 3 weeks old inoculated at the same time,



Bacterial Pustule of Soybean

Plate 3

Bacterium phaseoli sojense. (Photographs by JAMES F. BREWER)

A to D.--Sections of pustules 3 weeks old produced by inoculation; bacteria throughout. A.—Hypertrophy and hyperplasia; bacterial lesion (×), ×160. B.—Detail from A. ×900. C.—Pustule on lower leaf surface; a few bacteria penetrating normal tissue also. ×200. D.—Elongation of epidermal cells in pustules. ×260.

E.—*Bacterium phaseoli sojense* from 24-hour-old beef agar slant. Casares-Gil's flagella stain. ×2000.

in the same manner with the same culture, which, furthermore, produced good typical infections on soybean.

When previously passed through Phaseolus the soybean organism produced infections on bush string bean leaves and pods (Pl. 1, H, I; Pl. 4, B) in no way distinguishable from those caused by *Bact. phaseoli* EFS. (Pl. 1, J, to L).

INOCULATIONS WITH BACTERIUM PHASEOLI SOJENSE AFTER PASSAGE THROUGH PHASEOLUS

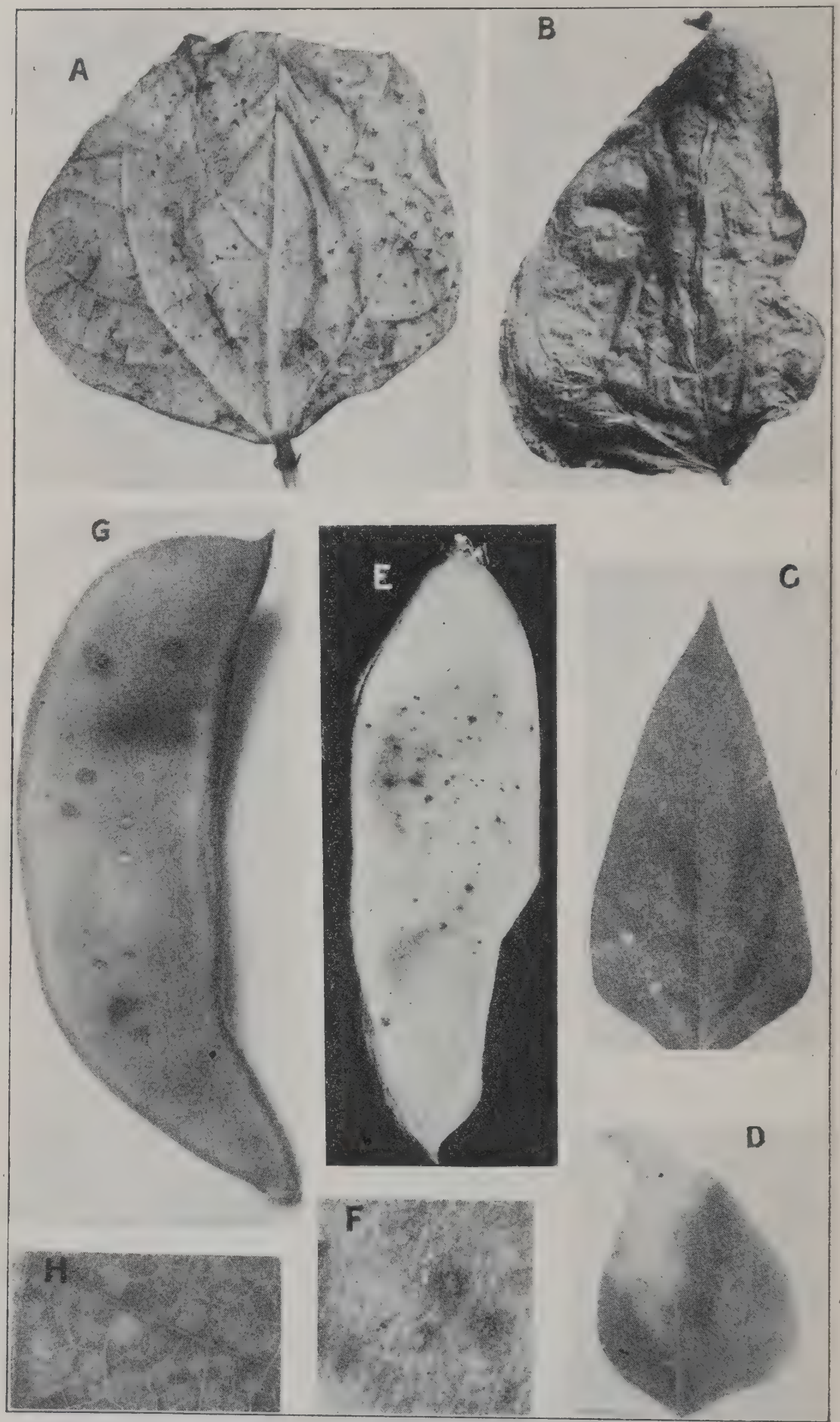
As it had occurred to the writer that the pathogenicity of *Bact. phaseoli sojense* for Phaseolus might be increased by its passage through members of that genus, parallel inoculations were made September 30, 1919, on King of the Garden Lima (3 weeks old), dwarf bush string bean (2 weeks old) and Hahto soybean (3 weeks old) with two cultures of *Bact. phaseoli sojense*, one (colony 3) plated May 26, 1919, from soybean, the other (colony 1) plated July 19, 1919, from a Lima leaf, but both descended originally from the same colony plated from the Texas material received in 1916.[5] The inoculations were made by spraying in inoculating cages in which the plants were kept moist for 72 hours. In addition to the above, six King of the Garden Limas (3 weeks old) were sprayed, three with each organism, and covered with paraffined bags for 72 hours. The results were as follows:

BACT. PHASEOLI SOJENSE

Inoculation experiment showing the effect of previous passage through Phaseolus

	COLONY 3 (FROM SOYBEAN)	COLONY 1 (FROM LIMA BEAN)
9th day	*Lima bean* (cage): Infection doubtful. Tiny red spots and distorted leaflets but no bacteria found in either on microscopic examination.	*Lima bean* (cage): Good infection, water-soaked spots.
	Lima bean (bags): Infection doubtful.	*Lima bean* (bags): Water-soaked spots. Bacteria in abundance.
	Dwarf bush string bean: Peppered with water-soaked spots.	*Dwarf bush string bean:* Water-soaked spots.
	Soybean: Typical infection, pustule stage.	*Soybean:* Infection doubtful.
11th day	*Lima bean* (cage): Still doubtful. Found no bacteria.	*Lima bean* (cage): Excellent infection. Spots becoming discolored.
	Lima bean (bags): A few tiny suspicious-looking spots.	*Lima bean* (bags): Excellent infection. Some of infected leaves are wilting and shrivelling.
	Dwarf bush string bean: Centers of spots becoming discolored.	*Dwarf bush string bean:* Excellent infection on a few leaves—discoloration beginning.
	Soybean: Center of pustules becoming brown.	*Soybean:* Infection doubtful.
15th day	*Lima bean* (cage): No typical spots.	*Lima bean* (cage): Spots increasing in size.
	Lima bean (bags): Infection doubtful.	*Lima bean* (bags): Spots increasing in size and surrounding tissue becoming yellow. Some shrivelling.
	Dwarf bush string bean: Spots have increased little, if any, in size.	*Dwarf bush string bean:* Conspicuous spots, increased in size; distortion of leaf due to infection of veins, shrivelling.
	Soybean: Typical infection.	*Soybean:* Infection doubtful.
18th day	*Lima bean* (cage): No typical spots but found bacteria in the distorted leaflets and pale green spots with tiny red centers and *isolated the parasite from both.*	
		Dwarf bush string bean: Some wilt also. *Isolated parasite* from petiole of wilted leaf.
27th day	*Lima bean* (cage and bags): No change.	*Lima bean* (cage and bags): Excellent infection in all. Shrivelled leaves.
	Dwarf bush string bean: No enlargement of spots which are still small and red. No yellowing. Small red spots full of bacteria. *Parasite isolated.* Photographed leaf. (Pl. 4, A).	*Dwarf bush string bean:* Some leaves shrivelled. Some have dropped off. Multitude of bacteria. *Parasite isolated.* Photographed leaf. (Pl. 4, B).
	Soybean: Typical infection	*Soybean:* No signs of infection

[5] These two colonies had passed through the following series of inoculations:
From Texas soybean: Colony e, Sept. 23, 1916 > soybean > Lima pod > soybean > { soybean > colony 3. / soybean > Lima colony 1.



Bacterial Pustule of Soybean

Plate 4

(For explanatory legend see p. 237)

BACT. PHASEOLI SOJENSE—Centinued

Inoculation experiment showing the effect of previous passage through Phaseolus—Continued

	COLONY 3 (FROM SOYBEAN)	COLONY 1 (FROM LIMA BEAN)
5th week	*Lima bean* (cage and bags): No noticeable increase in infection. The two worst infected leaves are much distorted but are neither yellowed nor shrivelled.	*Lima bean* (cage and bags): Striking infection. A number of leaves have dropped off, wholly browned and shrivelled or with only a small amount of green tissue remaining, as sometimes occurs in inoculations with *Bact. phaseoli* EFS. (Pl. 1, L). Others have large brown shrivelled areas bordered with yellow, involving one-half to three-fourths of the leaflet.
	Dwarf bush string bean: Spots somewhat larger than at time of previous observation but there has been no very noticeable spread of the infection.	*Dwarf bush string bean:* Conspicuous infection. Typical brown and shrivelled spots, bordered with yellow.
	Soybean: Spots have increased considerably in size.	*Soybean:* No signs of infection.
8th week	*Lima bean:* no secondary infections.	*Lima bean:* Conspicuous infections in every respect like those caused by *Bact. phaseoli* EFS.
	Dwarf bush string bean: No increase of symptoms. Infection is much more restricted than on plants inoculated with colony 1.	*Dwarf bush string bean:* Not distinguishable from infections caused by *Bact. phaseoli* EFS.
	Soybean: Spots enlarging somewhat. There has been good infection.	*Soybean:* No infection.

A repetition of the experiment with the same colonies corroborated the results set forth above.

A third set of inoculations was made with another pair of colonies[6] but the temperature of the hothouse (about 62° F.) was too low to favor infection.

In the parallel sets of inoculations here described, passage through Phaseolus seemed not only to increase the pathogenicity to Phaseolus but to destroy it for soybean. However, in two previous experiments excellent infections had been obtained on soybean by colony 1, August 6, 1917, through Lima (Pl. 1, E, F; Pl. 2, B, D); and in two later experiments with colonies isolated from bush string bean some scattering but typical infections were obtained on soybean (Pl. 1, G). With one of these colonies[7] excellent infections were produced on bush string bean, dwarf wax bean and dwarf bountiful string bean. No infection was obtained on dwarf wax or Early Refugee wax bean with the other colony,[8] but wilt was caused on dwarf bountiful string bean. (Though primarily causing leafspot, both *Bact. phaseoli sojense* and *Bact. phaseoli* EFS. may produce wilt if the organism invades the vascular system.)

In conclusion, however, it may be said that *in no case has the writer found the same colony of Bact. phaseoli sojense equally infectious to soybean and Lima or bush string bean.*

[6] From Texas soybean: Colony e, Sept. 23, 1916>soybean>Lima pod>soybean>soybean> { soybean>colony 5 / bush string bean>colony 12.

[7] From Texas soybean: Colony e, Sept. 23, 1916,>soybean>Lima pod>soybean>soybean>Lima>bush string bean>colony 6, Oct. 20, 1919.

[8] From Texas soybean: Colony e, Sept. 23, 1916 soybean Lima bean pod soybean soybean>bush string bean>colony 11, Dec. 5, 1919.

EXPLANATORY LEGEND FOR PLATE 4

Inoculations on Phaseolus with *Bacterium phaseoli sojense* (through soybean), *Bacterium phaseoli sojense* (through Lima bean), and *Bacterium phaseoli* EFS. for comparison. (Photographs by JAMES F. BREWER.)

A and B.—Comparative effect on bush string bean of *Bacterium phaseoli sojense* four weeks after inoculation with (A) organism through soybean; small spots; (B) organism through Lima bean: leaf shriveled ×1½.

C and D.—Comparative virulence to Lima of (C) *Bacterium phaseoli sojense* through soybean and (D) *Bacterium phaseoli* EFS. 18-day-old infections. ×1. C, tiny spots, no water soaking. D, excellent typical infection.

E to G.—Comparative effect on Lima pods of (E, F) *Bacterium phaseoli sojense* through soybean and (G) *Bacterium phaseoli* EFS. E, 3-weeks-old infection. ×1. F, detail from E. ×5 ca. G, 7-day-old infection. ×1.

H.—*Bacterium phaseoli* EFS. 11-day-old infection on Lima bean leaf, water soaking. ×5.

It is to be noted that the infection on all the varieties of Phaseolus inoculated with *Bact. phaseoli sojense* resembled that produced by *Bact. phaseoli* EFS. and in the best cases was not to be distinguished from it. There was even reddening of the veins in infected dwarf bountiful string bean, but *in no case were pustules produced.* Wilt was occasionally observed in infections on Phaseolus. Microscopic examination of the petiole of a wilted bush string bean leaf showed that the bacteria were present in the vessels but not confined to them.

COMPARATIVE INOCULATIONS ON BEANS AND SOYBEANS WITH BACTERIUM PHASEOLI EFS. AND BACTERIUM PHASEOLI SOJENSE (THROUGH SOYBEAN)

Not only is *Bact. phaseoli* EFS. much more infectious to Phaseolus than the soybean organism, but thus far, with the exception of a single experiment, it has been entirely nonpathogenic to soybean seedlings or older plants, when inoculations have been made by any of the methods so successful in producing infection on Phaseolus with *Bact. phaseoli* EFS. or soybean by inoculation with *Bact. phaseoli sojense.* Infection was obtained when a modification of these methods was employed, that is, when the leaves were rubbed gently between thumb and forefinger after spraying. In addition to spraying and pricking inoculations in inoculation cages, an attempt was made to produce the disease by so placing healthy young soybeans among very badly diseased Lima beans that their leaves would come in contact with the bacterial ooze from the leaves of the latter, and by watering the plants in such a way that the bacteria would be washed down upon the soybeans. With the single exception noted above, all of these inoculations failed. There were 24 of these experiments, made in every month except May and November.

The history of the exceptional inoculation experiment is as follows: Eight pots of Mammoth Yellow soybean seedlings grown from seeds treated with concentrated H_2SO_4 and planted September 15, 1920, were sprayed at 4 p. m. October 4 with a 2-day-old culture of what proved later to be a very virulent colony of *Bact. phaseoli* EFS. (colony 6, August 24, 1920, isolated from string bean leaves). The seedlings ranged in size from those just appearing above the ground to plants 8 or 9 inches high. Some were pricked as well as sprayed. The plants were sprayed again the following day at 10 a. m. and 4 p. m. and were kept in a moist atmosphere for 45 hours. On October 15, 11 days after inoculation, there were a few scattering brown spots, some of them marginal. These were not associated with the pricks. There were no pustules and the spots did not resemble infection by *Bact. phaseoli sojense* nor did microscopic examination reveal the presence of bacteria. Plates were poured, however, and *Bact. phaseoli* EFS. isolated, but although the plates were inoculated very heavily only seven colonies appeared. Two of these showed the radiating lines, and two the internal concentric striae both illustrated in Plate 7, B and J. In other spots examined later a few scattering bacteria were found. The browning may have been partly due to asphyxiation; yet in both this experiment and in some described later *Bact. phaseoli* EFS. was plated from the browned areas.

This very slight evidence of the pathogenicity of *Bact. phaseoli* EFS. for soybean led to some experiments which resulted in very good infections on soybean, but the conditions were quite abnormal. The inoculation experiment described below gives a fair idea of the typical differences usually found between *Bact. phaseoli sojense* and *Bact. phaseoli* EFS. in their pathogenicity to Lima bean and soybean:

Three rapidly growing Lima bean plants (Burpee's Fordhook Lima) just beginning to put forth tiny flower buds and two Hahto soybean seedlings 8 to 12 inches high were inoculated June 5, 1919, by spraying with *Bact. phaseoli* EFS. In other experiments many more soybeans were inoculated, but with the same results. For comparison eight large Hahto soybeans and two seedlings of the same variety about 8 inches high, also one Lima bean plant with several pods and blossoms, were inoculated with the soybean organism (from soybean). In other experiments more Lima beans were used, but with the same results. Two colonies of each organism were used, and both sets of plants were put into inoculation cages and kept in a very hot, moist atmosphere for two days. The results were as follows:

	BACTERIUM PHASEOLI SOJENSE (THROUGH SOYBEAN)	BACTERIUM PHASEOLI EFS.
8th day	*Soybean* seedlings: pale green spots, pustules. Large plants: no signs of infection. *Lima:* No signs of infection.	*Soybean* seedlings: No signs of infection. *Lima:* Excellent infections on all plants. On some of the younger leaves there is scarcely any sound tissue and the leaves are distorted. The leaves have a pale green mottled appearance on the surface and are a mass of water-soaked spots underneath.
11th day	*Soybean:* No noticeable change. Both colonies infectious. *Lima:* No signs of infection.	*Soybean* seedlings: No signs of infection. *Lima:* Worst infected leaves are rapidly shrivelling. Spots brown and surrounding tissue is turning yellow.
13th day	*Soybean* seedlings: Spots larger and considerably more conspicuous. Larger plants: No signs of infection. *Lima:* No signs of infection.	*Soybean* seedlings: No signs of infection. *Lima:* Bacterial ooze on the infected leaves. The flowering has been much retarded, buds still very tiny while checks are in full flower. Brown spots coming on stems of all plants.
16th day	*Soybean:* No infection on older plants. *Lima:* No signs of infection.	*Soybean* seedlings: No infection. *Lima:* Distortion of many of the younger diseased leaves where enough contiguous sound tissue was left to continue to grow normally while the diseased area could not. Many yellowed leaves. Many leaves dropping off.
18th day	*Soybean:* No infection on older plants. *Lima:* No signs of infection.	*Soybean:* No signs of infection. *Lima:* In some of the leaves practically all the chlorophyll is gone—only isolated patches of green. The rest of the leaf is yellow, not brown or shrivelled or dry.
19th day	*Soybean:* No infection on older plants. *Lima:* No signs of infection.	*Soybean:* No infection. *Lima:* Excellent stem infections—external brown spots filled with bacteria. Flower buds not opening. Many buds falling off.
5th week	*Soybean:* Infection on older plants also. *Lima:* A few infections. These are like typical *Bact. phaseoli* EFS. infections. Multitudes of bacteria.	*Soybean* seedlings: No infection.
6th week	*Lima:* Many water-soaked spots on some of the leaves. This water-soaking is less noticeable to the naked eye than that in young spots on Lima produced by *Bact. phaseoli* EFS. Slight elevation of the epidermis but no real pustules.	*Soybean* seedlings: No infection.
7th week	Soybean photographed (Pl. 2, C).	

Later comparative inoculations on Lima bean in which younger plants were inoculated with the two organisms produced similar results (Pl. 4, C, D). Infection with the soybean organism (through soybean) took place very slowly. The water-soaking was usually absent or much less conspicuous than in infections with *Bact. phaseoli* EFS. and sometimes the only signs of infection for weeks were tiny pale green spots with or without red centers. Usually, however, a few spots resembling typical *Bact. phaseoli* EFS. infections appeared weeks after inoculation.

In both these sets of comparative inoculations with *Bact. phaseoli sojense* (through soybean) and *Bact. phaseoli* EFS. the difference in virulence for Lima bean was very striking throughout the experiments.

INFECTION PRODUCED ON SOYBEAN SEEDLINGS BY BACTERIUM PHASEOLI EFS.

The first very successful infections on soybeans with *Bact. phaseoli* EFS. were obtained on the cotyledons of Ito San soybeans germinated and inoculated in a sterile Petri dish damp

chamber. The seeds were treated with formaldehyde by the presoak method [9] described by Braun (*1*) and put into the damp chamber November 24, 1920. Two days later, having germinated, they were inoculated by spraying with a water suspension of a 2-day old culture of *Bact. phaseoli* EFS. The following day they were sprayed again. No leaves were yet visible. On the third day after inoculation some of the leaves were beginning to appear and the seedlings were transferred to large moist chambers.

On November 30 there were no signs of infection and five of the seedlings (Nos. 16 to 20) were transferred to Sachs' nutrient solution and placed under a bell jar in a south window in the laboratory. On December 2 they were reinoculated by spraying. The leaves were just beginning to be visible between the cotyledons in some cases; in others they were beginning to push out.

Eight days after the first spraying in the Petri dish the cotyledons of many of the seedlings remaining in the damp chamber had water-soaked spots and bacterial ooze therefrom (Pl. 7, N, O). In some cases the seed coat was still firmly attached and the spots were visible through it. Seven of these seedlings with infected cotyledons (Nos. 45–51) were transferred to Sachs' solution and placed under a bell jar in a north window in the laboratory. They were sprayed heavily with sterile H_2O in an attempt to spread the infection by scattering the bacteria oozing from the spots.

On December 9 seedlings Nos. 16 to 20, reinoculated December 2 (two days after their transfer to Sachs' solution), showed a pronounced browning and curling of the leaf tips or margins (like Pl. 7, P). Five days later *Bact. phaseoli* EFS. was isolated from the browned curled margin of a leaf of No. 20 in which microscopic examination showed the bacteria present in abundance.

Also on December 9 seedlings Nos. 45 to 51, transferred to Sachs' solution on December 4 but not reinoculated, were all very much stunted. The main shoot was shriveling in Nos. 48 to 51 and there were irregular brown spots on the leaves of the other three (Nos. 45 to 47). *Bact. phaseoli* EFS. was reisolated December 11 from dark brown spots on the cotyledons of plant No. 46. On December 31, all the plants transferred to Sachs' solution were dead, doubtless partly because of the abnormal conditions under which they had been grown.

Similar results were obtained on Ito San soybean seed treated with sulphuric acid [10] and inoculated by spraying in a Petri dish damp chamber immediately after germination therein.

In a third experiment presoaked soybean seeds of the Ito San variety, treated with formaldehyde, were germinated in a damp chamber and transferred as soon as the seedlings had made sufficient growth to Sachs' solution and inoculated with *Bact. phaseoli* EFS. 8 days after the sowing of the seed in the damp chamber. In some cases the leaves had not begun to push out, but the cotyledons were spreading and the culture was sprayed between them upon the folded leaves at 2.15 p. m. They were placed under a low bell jar in a south window of the laboratory; at 4.15 they were sprayed again and twice the next day, at 10.20 a. m. and 4.30 p. m. The moisture held well for 48 hours. The low bell jar was then replaced by a taller one and this was removed 2 days later. A week after the inoculation there was a curling and yellowing or browning of the margin of many of the first leaves (like Pl. 7, P), and *Bact. phaseoli* EFS. was isolated from two of them. No infection of cotyledons was observed.

Ito San soybean seedlings grown like the preceding but taken to the greenhouse, after their transfer to Sachs' solution, and there inoculated eight days after the seed was sown in the damp chamber, became similarly infected. One of these photographed eight days after inoculation, is shown in Plate 7, P.

These experiments were repeated several times and always resulted in infection. *Bact. phaseoli* EFS. was repeatedly isolated both from the cotyledons and the brown curled leaf margins. *No pustules were ever produced.*

Another attempt was made on December 10, 1920, to produce infection with *Bacterium phaseoli* EFS. on young seedlings of Ito San and Mammoth Yellow soybeans growing in pots in the greenhouse, but the only positive results were obtained on 11 Ito San seedlings, the leaves of which had been rubbed

[9] H_2O for 10 minutes in bags of double thickness surgeon's gauze; moist chamber (still in bags), 6 hours; 1:300 formaldehyde for 15 minutes; moist chamber (wet with 1:300 formaldehyde), 45 minutes; spread out to dry overnight.

[10] H_2SO_4 (concentrated) for 1 minute; H_2SO_4 poured off and sterile water added; sterile water added and poured off several times.

gently between the thumb and forefinger after spraying with the bacterial suspension. There were 23 Ito San and 61 Mammoth Yellow soybean plants used in the experiment. The Ito San seed had been treated with formaldehyde and germinated in a damp chamber, then planted in pots. The plants had developed the first pair of leaves when inoculated. The Mammoth Yellow seed was untreated and germinated in the soil. The cotyledons were just beginning to spread, showing a leaf shoot at the time of inoculation.

The plants were kept moist in inoculating cages, bell jars, or paraffined bags for 28 hours after spraying with *Bact. phaseoli* EFS. On the rubbed leaves of the 11 infected Ito San seedlings were narrow brown streaks and pale green areas, from the former of which the parasite was reisolated. Twelve other Ito San seedlings and 32 Mammoth Yellow seedlings were in the same inoculating cage, but no brown streaks appeared nor did they ever show any signs of infection. In this case it seems reasonable to conclude that the brown streaks were due to infection rather than to asphyxiation.

Another method which proved successful was that of dipping the seed of Ito San soybean treated by the H_2SO_4 method, in a water suspension of the organism and germinating it in a damp chamber. The parasite was reisolated from a water-soaked cotyledon on one of these soybeans. For this type of inoculation *Bact. phaseoli* EFS. isolated from soybean was used.

INOCULATION OF SOYBEAN AND LIMA BEAN WITH BACTERIUM PHASEOLI EFS. AFTER PASSAGE THROUGH SOYBEAN AND VELVET BEAN

On the assumption that passage through soybean might increase the pathogenicity of *Bact. phaseoli* EFS. to soybean, spray inoculations were made upon seedlings of this plant and of Lima bean in inoculation cages. But passage of *Bact. phaseoli* EFS. through soybean (*Glycine hispida*) or Georgia velvet bean (Stizolobium) did not increase its virulence for soybean, nor, on the other hand, did passage through soybean decrease its pathogenicity to Lima bean, as evidenced by the fact that the worst cases of infection which the writer has ever seen were produced on King of the Garden and Fordhook Bush Lima sprayed in the hothouse in February with *Bact. phaseoli* EFS. isolated from inoculated soybean. The high temperature maintained in this house, where the thermometer rarely fell below 75° F. during the night, and for days reached 103 to 110° in the middle of the day, was in a very large measure responsible for this. The difference in the severity of the infection on the plants in this house and on plants of the same variety and age, inoculated at the same time with the same culture, but kept in a house with a temperature ranging from 64° to 72° F. the greater part of the time, was striking in the extreme. In the hotter house infection was much more abundant and progressed farther and much more rapidly. One of the worst infected plants collapsed entirely. The whole top was dead and the stem was withered and full of bacteria. The infection of the stem extended all the way down to the ground from the first pair of leaves, a distance of 6 inches. There were drops of yellow ooze from the stem when squeezed. These plants were inoculated when they had only the first pair of leaves.

CULTURAL CHARACTERS

In all cultural work on the soybean organism parallel tests were made with *Bact. phaseoli* EFS., but inasmuch as with a few exceptions no greater differences were found than might exist between two colonies of the same organism, the reader is referred to Smith's published descriptions of the cultural characters of *Bact. phaseoli* EFS. (*15*, *16*, *17*, *20*, *p. 285–287*) and space will be given here only to the differences found and to some additional facts not hitherto recorded.

The best medium found for the soybean organism is fresh steamed potato cylinders. *Bact. phaseoli sojense*, like *Bact. phaseoli* EFS., makes a very copious growth on this medium, consuming practically all the starch, and so filling the 2 cc. or more of water around the cylinders with slime that it becomes a solid yellow mass and does not flow when the tubes are turned upside down. The writer has never failed to obtain this characteristic growth if the potato cylinders were fresh, but even a slight drying out of the upper part of the cylinder may alter the character of the growth.

Much of the cultural work was done before P_H determinations were made in this laboratory, and "approximately" after the P_H reading means that the latter was worked out from the Fuller's scale reading according to the formula of Quirk and Fawcett (*12*, *p. 20*).

The descriptions which follow relate equally to *Bact. phaseoli sojense* and *Bact. phaseoli* EFS. unless otherwise noted:

BEEF AGAR SLANTS.—(+15 Fuller's scale, P_H 6.8, actual determinations). Moderate pale yellow, flat, filiform, smooth, glistening, translucent growth in 24 hours. No distinctive odor. May become slightly viscid. Growth increases somewhat but is never copious.

BEEF AGAR STABS.—(+15 Fuller's scale, approximately P_H 6.8).

(All the beef agars used were made with beef infusion +1 per cent Difco peptone.) Best growth at top. Surface growth good, nearly covering surface of agar, nailhead slightly convex. Line of puncture filiform. Never very much growth at bottom of the stab.

GILTNER'S CONGO RED [11] AGAR.—Very thin, colorless, blisterlike growth in 24 hours. In 3 to 4 days there is a thick, smooth, wet-shining growth along the line of the stroke which has absorbed the red but has a slightly yellowish tinge. In six days the surface of the slant is nearly covered by a copious growth. Agar becomes purplish in 2 to 6 weeks, a decided contrast to the check. An excellent medium for this organism.

SYNTHETIC AGAR.—(Like the preceding without the Congo red). Excellent growth as in the above. Very little yellow pigment. Pigment best seen by oblique light.

LITMUS DEXTROSE AGAR.[12]—Good growth along the path of the streak in 2 days. No acid or alkali produced in 4 days and no reduction of the litmus. Considerable alkali produced in 3 weeks.

LITMUS LACTOSE AGAR.[13]—Thin growth covering the surface of the slant in 2 days. No change in the color of the agar in 3 to 4 days. Slight bluing in 5 days. Decided bluing in 8 to 10 days. Growth greenish yellow and not copious (10 days). In 3 to 4 weeks the blue of the agar had a decidedly greenish tinge. There was never any reduction of the litmus. (Under observation 4½ months.)

LÖHNIS SOIL EXTRACT AGAR.[14]—Barely visible growth in 24 hours. Still a very scanty growth in 5 weeks.

POTATO AGAR [15] PLUS 10 PER CENT DEXTROSE.—Good growth along the path of the streak in 24 hours. Excellent, smooth, yellow, wet-shining growth in 2 days. All the potato starch consumed by the sixteenth day.

WHEY AGAR.[16]—Good to copious, wet-shining, yellow growth, sometimes piled up along the streak in 3 days. Surface of slant nearly covered in 6 days. In one set of 9-day-old cultures *Bact. phaseoli sojense* was amber yellow and *Bact. phaseoli* EFS. primuline yellow (*13*).

LOEFFLER'S BLOOD SERUM.—Excellent growth. Liquefaction begins in 6 to 9 days, good but not complete liquefaction in 2½ months.

NUTRIENT GELATIN.—(+10 to +12 Fuller's scale, P_H 7.2 to 7, actual determinations.) Beef infusion bouillon, 1 per cent peptone, 10 per cent gelatin. Gelatin liquefied promptly, somewhat more rapidly by *Bact. phaseoli sojense* than by *Bact. phaseoli* EFS. Liquefaction begins in 2 to 3 days and is usually stratiform.

BEEF BOUILLON.—(+12 to +15 Fuller's scale, P_H 7.2 to 6.9, actual determinations.) Thin to moderate clouding in 24 hours, with rolling clouds on shaking, partial rim, no pellicle or sediment. Flocculence and heavy yellowish rim stringing down into the liquid in 4 or 5 days. In 3 to 4 weeks there is a partial or complete pellicle which may be thin or heavy, stringing down into the liquid and shaking down easily; and the culture medium is clearing. Viscid yellow precipitate in cultures two or more weeks old.

FERMENTATION TUBES.—Tested the organism in 1 per cent solutions of saccharose, maltose, mannit, glycerin, dextrose and lactose in 1 per cent Difco peptone water. No gas in any. Good clouding in open end in all. Faint clouding in closed end in lactose in 7 days. Titration at the end of 18 days showed an increase of acid in the cultures—most in maltose, saccharose, and dextrose.

[11] 1,000 cc. distilled water; 10.0 gm. saccharose; 1.0 gm. dipotassium phosphate; 0.2 gm. magnesium sulphate; 15.0 gm. agar flour; 0.1 gm. Congo red. Water and all salts steamed for 30 minutes. Congo red then added. Filtered through cotton and tubed. Autoclaved 15 minutes at 110° C.

[12] 1 per cent beef infusion agar+1 per cent Difco peptone+litmus+1 per cent dextrose.

[13] 1 per cent beef infusion agar+1 per cent Difco peptone+litmus+1 per cent lactose.

[14] 150 gm. soil; 1,000 cc. distilled water; trace calcium oxide; 0.5 gm. dipotassium phosphate; 10.0 gm. mannite; 15.0 gm. agar; excess calcium carbonate.

[15] 750 cc. potato juice; 10 per cent dextrose; 2 per cent agar. Steamed 45 minutes. Filtered through cotton. Autoclaved 15 minutes at 110° C.

[16] Fresh milk boiled gently for 5 minutes, 20 per cent HCl added drop by drop till all casein was precipitated. (An excess of acid is to be avoided.) Coagulum removed by straining through cheesecloth and the whey made exactly neutral to litmus (neutral litmus) with 20 per cent solution of sodium hydroxide. To each liter of whey was added: 300 cc. distilled water; 3 gm. gelatin; 15 gm. cane sugar; 15 gm. peptone; 15 gm. agar. Steamed 30 minutes.

USCHINSKY'S SOLUTION.—Faint to moderate clouding. Medium in old cultures (3½ to 4 months) is the color of beef broth. Cultures of *Bact. phaseoli sojense* sometimes become reddish brown.

LITMUS MILK.—Reduction of litmus beginning in 4 to 16 days, complete in 5 to 19 days (occasionally not complete in 3 weeks). Otherwise as recorded in descriptions cited.

MILK PLUS METHYLENE BLUE.—All color disappears in 1 to 2 days. Decided yellowish cast to whey and coagulum in 3 weeks.

SOYKA'S RICE MILK.—Bright yellow growth.

TOLERATION OF ACID AND ALKALI [17]

This work was done in conjunction with Quirk and Fawcett in their work on "Hydrogen-ion concentration vs. titratable acidity in culture mediums" (*12, p. 21, 50, 54*).

Tests of acid and alkali toleration were made in 1 per cent peptone beef broth (hot infusion A (*12, p. 21*)), to which NaOH or HCl was added. Growth occurred in 24 hours in 0 to +17 Fuller's scale (8.1 to 6.5 P_H)[17] and in 72 hours *Bact. phaseoli* EFS. had grown in −3 to +28 Fuller's scale (8.4 to 5.7 P_H)[17], and *Bact. phaseoli sojense* in 0 to +28 (8.1 to 5.7 P_H).[17] In 2 weeks both had clouded the broth which titrated, at the time of inoculation, −6 Fuller's scale (8.7 P_H).[17] A titration of check tubes, however, showed that the acidity of this medium had increased considerably upon standing. At the end of a week the check titrated −3 Fuller's scale (8.3 P_H)[17] and at the end of 2 weeks, at which time growth was noted, the check titrated −1 Fuller's scale (8.2 P_H).[17] There was still no clouding of +32 Fuller's scale (5.238 P_H).[17] A titration of a check tube showed that the acidity had changed very little on standing 2 weeks. The check titrated +33 Fuller's scale (5.2, 5.16 P_H).[17]

TOLERATION OF SODIUM CHLORIDE

Slight growth in beef bouillon neutral to phenolphthalein plus 4 per cent NaCl. Transfers made from the cultures to potato on the thirtieth day produced no growth.

PRODUCTION OF AMMONIA AND INDOL

Ammonia was produced in beef bouillon (+12 Fuller's scale, P_H 5.8, actual determinations) tested on the twenty-second day with Nessler's solution. No indol was found in Uschinsky plus peptone. The sodium nitrite-sulphuric acid test was made on the twenty-first day, but there was no pink reaction even upon heating.

VARIETAL DIFFERENCES BETWEEN BACT. PHASEOLI SOJENSE AND BACT. PHASEOLI EFS. IN PLATE CULTURES

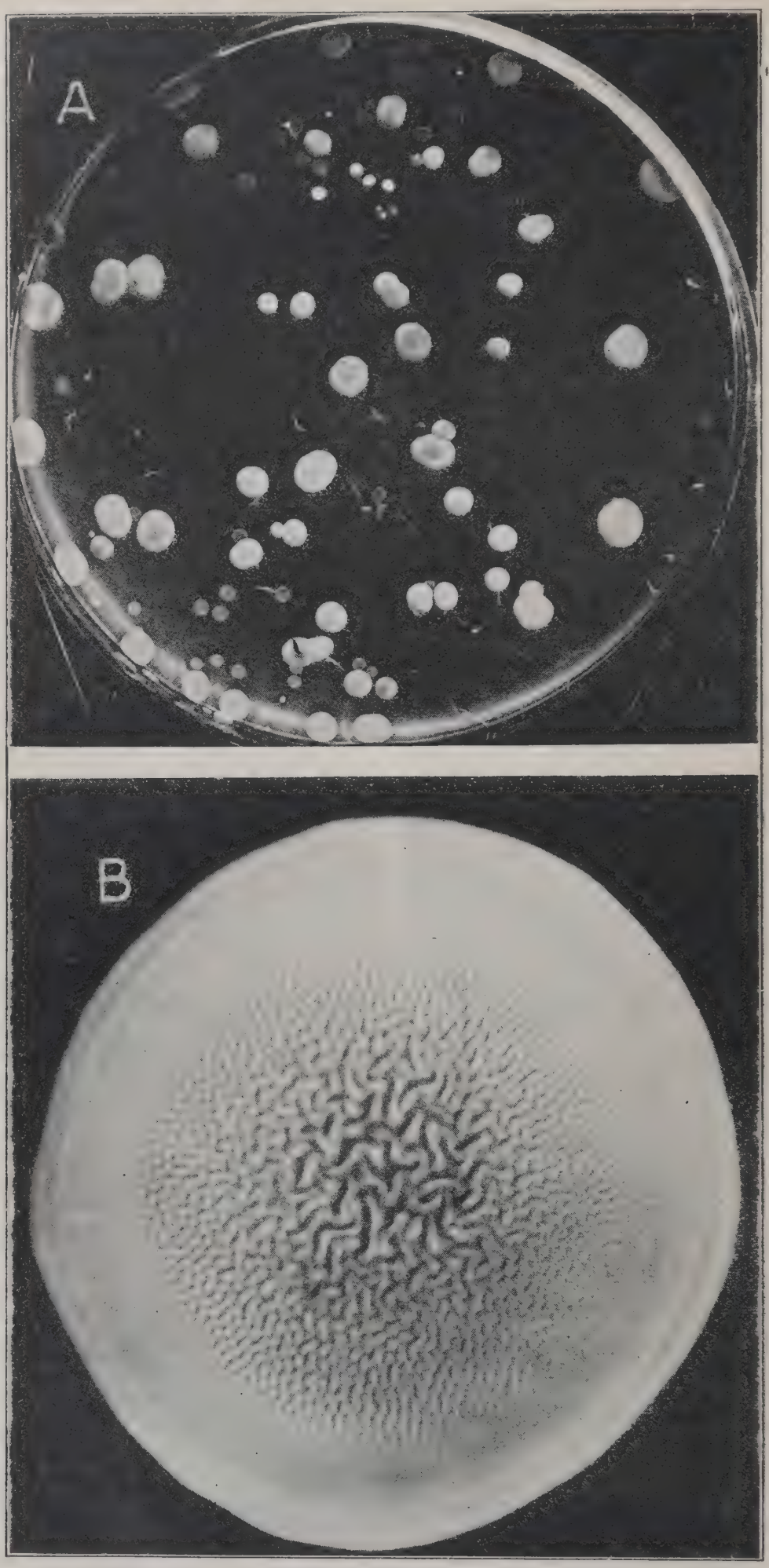
In the preceding tests *Bact. phaseoli sojense* was not distinguishable culturally from *Bact. phaseoli* EFS. There have, however, been some interesting differences in the behavior of the colonies on beef-agar plates.

COLONIES OF BACT. PHASEOLI SOJENSE ON 1 PER CENT BEEF AGAR (MADE WITH BEEF INFUSION+1 PER CENT PEPTONE AND 2 PER CENT AGAR) PLATES AT ROOM TEMPERATURE (PL. 5 AND 6)

Brief mention was made in a previous paper (*9*) of certain internal markings very commonly, though not universally, appearing in the colonies of *Bact. phaseoli sojense* which serve to distinguish it in a measure from *Bact. phaseoli* EFS. inasmuch as they have been observed but twice in the innumerable isolations made from the Phaseolus leafspot caused by the latter organism. When the colonies of *Bact. phaseoli sojense* first appear (in 2 or 3 days, rarely 4 or 5) they are pale yellow, round, smooth, wet-shining, slightly convex, with entire margins and no internal markings. At this time they are in no way distinguishable from *Bact. phaseoli* EFS. They also become a deeper yellow with age like the former, but in 5 to 7 days there very commonly appear in the surface colonies on the more thinly sown plates the aforesaid markings which are visible for 1 to 2 weeks (rarely 3 weeks). They are wholly internal, the surface of the colony remaining smooth. They are usually seen only by oblique transmitted light and a hand lens (Zeiss ×6) but they are sometimes so distinct that they are plainly visible by reflected light and the naked eye (Pl. 5, A).

There is more than one type of these markings, but by far the most common and the one which distinguishes *Bact. phaseoli sojense* from *Bact. phaseoli* EFS. is one which the author has termed *internal convolutions* (Pl. 5, B). There are various modifications of this type (Pl. 6, A, C, D, F). When they

[17] Potentiometer determinations.



Bacterial Pustule of Soybean

Plate 5

Bacterium phaseoli sojense. Colonies with internal convolutions. (Photographs by JAMES F. BREWER)

A.—Beef agar plate from small red spots on bush string bean, 3 weeks after inoculation (Pl. 4, A). Convolutions visible to naked eye. 8 days old. ×1.

B.—Plated from pale green spots with red centers on Lima bean leaf 12 days after inoculation. Colony 13 days old. ×6 ca.

were first observed (Pl. 6, A) they were called *reticulations*, but this term does not as well describe the appearance in most of the later isolations as does "convolutions."

Another rather common type of markings is "radiating lines." These are sometimes very distinct (Pl. 6, B). sometimes much less striking (Pl. 6, G).

A third and much rarer type for *Bact. phaseoli sojense* is that of "concentric striæ" (Pl. 6, E).

Very often more than one of these types is found on the same plate and, on the other hand, the author has never seen a plate in which there were not some surface colonies with no markings at all. The markings have never been observed on very thickly sown plates.

Thus far no explanation of the appearance or nonappearance of these markings has been found. The following observations have been made:

AGE AND TITRATION OF THE BEEF AGARS USED.—The variations in the age and reaction of the agar apparently have no effect on the type of colony produced.

Convoluted colonies.—On agar 2 to 16 days old at the time of inoculation and titrating +10 to +15 Fuller's scale (P_H7.2 to P_H6.7 approximately). These figures refer to the titration immediately after the final sterilization of the agar. +10 to +15 Fuller's scale beef agar may become 2° (Fuller's scale) more acid upon standing 2 weeks.

Colonies with radiating lines.—On agar 2 to 16 days old at the time of inoculation and titrating +11 to +15 Fuller's scale (P_H7.1 to P_H6.7, approximately).

Concentrically striated colonies.—On agar 4 to 9 days old at the time of inoculation, titrating +14 to +15 Fuller's scale (P_H6.8 to P_H6.7 approximately).

Plates containing no colonies with markings of any kind.—On agar 1 day to 3 months old at the time of inoculation, titrating +14 to +15 Fuller's scale (P_H6.8 to P_H6.7, approximately).

The fact that both marked and unmarked colonies, and colonies with various types of markings, appeared on the same plates would indicate at the outset that in these experiments the variations in the agar used had little, if any, influence. The marked colonies were in all portions of the plates (Pl. 5, A), hence a varying depth of agar because the plates were not entirely flat on the bottom could not be the cause of colony variation.

LENGTH OF TIME IN THE PLANT (THAT IS, TIME OF INOCULATION TO TIME OF ISOLATION).—All types were found in both young and old infections:

Convoluted colonies.—Plated from infections 15 days to 2½ months old.

Colonies with radiating lines.—Plated from infections 8 days to 3 months old.

Concentrically striated colonies.—Isolated from infections 15 days to 2½ months old.

Plates containing no colonies with markings of any kind.—Poured from infections 8 days to 2½ months old.

All types have been obtained in the plates poured from artificial infections with *Bact. phaseoli sojense* on both soybean and Phaseolus.

GENEALOGY OF COLONIES WITH INTERNAL MARKINGS.—There is a tendency for the variously marked colonies to remain true to type after passage through the plant.

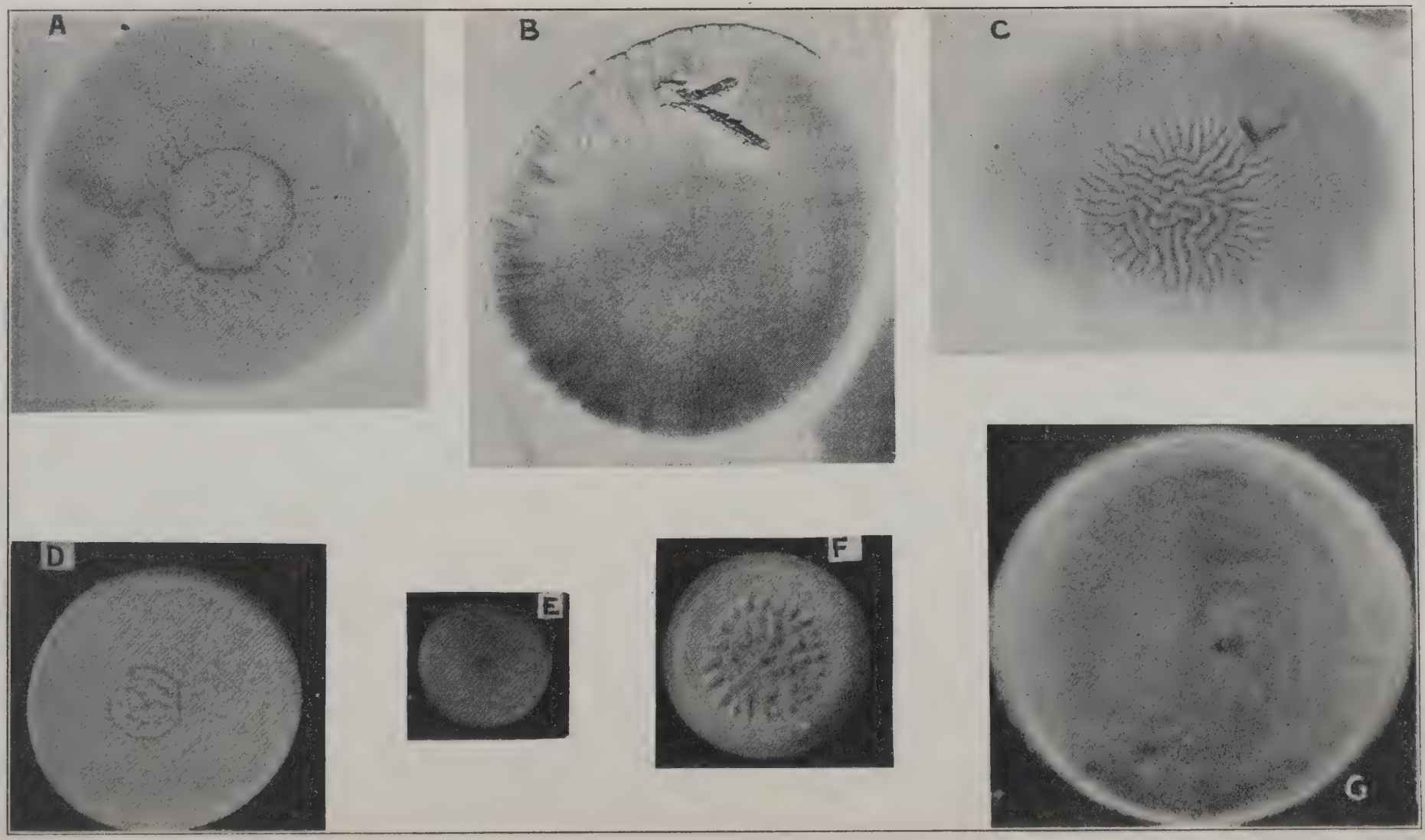
Convoluted colony 3, May 26, 1919 (Pl. 6, C).—Out of seven platings from infections produced by this colony, six contained convoluted colonies. The six platings were from four (possibly six) different plants of soybean, Lima and bush string bean. Three of these platings also contained concentrically striated colonies (Pl. 6, E), and one contained only colonies with no markings at all.

Convoluted colony 1, August 6, 1917 (like Pl. 6, A).—Five of eight platings from plants inoculated with this colony gave convoluted colonies. The five platings were from four (possibly five) different plants of soybean. Two sets of plates contained only unmarked colonies and one contained those with radiating lines (Pl. 6, B).

Concentrically striated colony 11, December 5, 1919 (like Pl. 6, E).—Two sets of plates were poured from infections produced by this colony. In both cases striated colonies were present and in one set of plates convoluted colonies also. The plates were poured from two different plants, soybean and string bean.

Colony with radiating lines.—Colony 1, April 26, 1919 (Pl. 6, B). From the single set of inoculations made with this colony only colonies of the same type and unmarked colonies have been isolated.

Colony with no markings.—Colony 6, October 20, 1919. Colonies of all the types above mentioned appeared in two sets of plates from infections with this colony. In one set of plates from soybean, convoluted, striated, and unmarked colonies were present; in the other, from wax bean, colonies with radiating lines and unmarked ones were present.



Bacterial Pustule of Soybean

Plate 6

(For explanatory legend, see p. 247)

TYPE OF COLONY IN RELATION TO VIRULENCE.—Typical infections have been obtained with all the types of colonies described. Apparently there is no relation between the colony markings and the degree of virulence.

COLONIES OF BACTERIUM PHASEOLI EFS. ON 1 PER CENT BEEF AGAR PLATES AT ROOM TEMPERATURE (PL. 7).

The type of colony most commonly appearing in isolation plates of *Bact. phaseoli* EFS. is round, smooth, wet-shining, slightly convex with thin, clear, colorless regular margins (Pl. 7, D). Frequently, however, colonies with coarse or fine concentric striæ are present (Pl. 7, A, J) and more rarely colonies with distinct radiating lines (Pl. 7, B, C); as in the case of the soybean organism, these markings are internal. Occasionally there is a lobed and fluted colony (Pl. 7, I), and there are still other types now and then (Pl. 7, K to M). In colony 7, May 9, 1919 (Pl. 7, L), the center was considerably depressed, as was that of most of the colonies on the same plate. Only twice have the colonies with internal convolutions so characteristic of *Bact. phaseoli sojense* been observed (Pl. 7, C).

As in the case of *Bact. phaseoli sojense*, the different types of colonies sometimes appeared on the same plate (Pl. 7, A, B). All colonies in Plate 7 produced infection except K and M, which were not tested. One set of comparative inoculations is illustrated in Plate 7, E to H. On August 12, 1918, Lima bean pods were sprayed with water and smeared with slime from potato cultures of August 3 of four types of colonies—striated, with radiating lines, convoluted, and unmarked (Pl. 7, A to D). The pods were then wrapped in oiled paper. When the paper was removed, August 19, there were water-soaked spots (Pl. 4, G), and on August 31, 19 days after inoculation, the pods were badly infected, as shown in Plate 7, E to H. In this experiment the striated and unmarked colonies were by far the most infectious, but in other inoculations the type with radiating lines has also been very virulent. The convoluted type came from a much older isolation than the others. It was isolated in Idaho in 1914, and the others in 1917. This may explain its lesser degree of virulence, since it was formerly very infectious.

MORPHOLOGY OF BACTERIUM PHASEOLI SOJENSE

Short rods with rounded ends, single or in pairs 1.4 to 2.3 μ × 0.5 to 0.9 μ. Actively motile, 1–2 polar flagella. Rods from water suspension of beef agar slants 1 to 3 days old stained with Casares-Gil's (Pl. 3, E) and van Ermengem's flagella stains. Smith describes *Bact. phaseoli* EFS. as one-flagellate (*16*).

LONGEVITY

IN CULTURES.—*Bacterium phaseoli sojense* lives on potato at room temperature three to five months provided inoculation is made on freshly made cylinders. Stock cultures in the ice box have been alive one year and four months on both potato and beef agar (stabs) in small-bore tubes (1½ cm. diameter). On the other hand, beef agar stock cultures in tubes 2 cm. in diameter did not live six months in the ice box. *Bact. phaseoli* EFS. has lived 1½ years on beef agar (small-bore tubes) in the ice box but has died in three months in the ice box when growing in beef agar in the large-bore tubes.

IN THE PLANT.—*Bact. phaseoli sojense* has been plated from soybean leaves kept dry in the laboratory for eight months, and colonies so obtained were very infectious. Very few of the organisms were alive, however, and it is often difficult or impossible to isolate the parasite from such material.

RETENTION OF VIRULENCE

Bacterium phaseoli sojense does not lose its virulence readily. Excellent infections have been obtained with a colony 1½ years after its isolation.

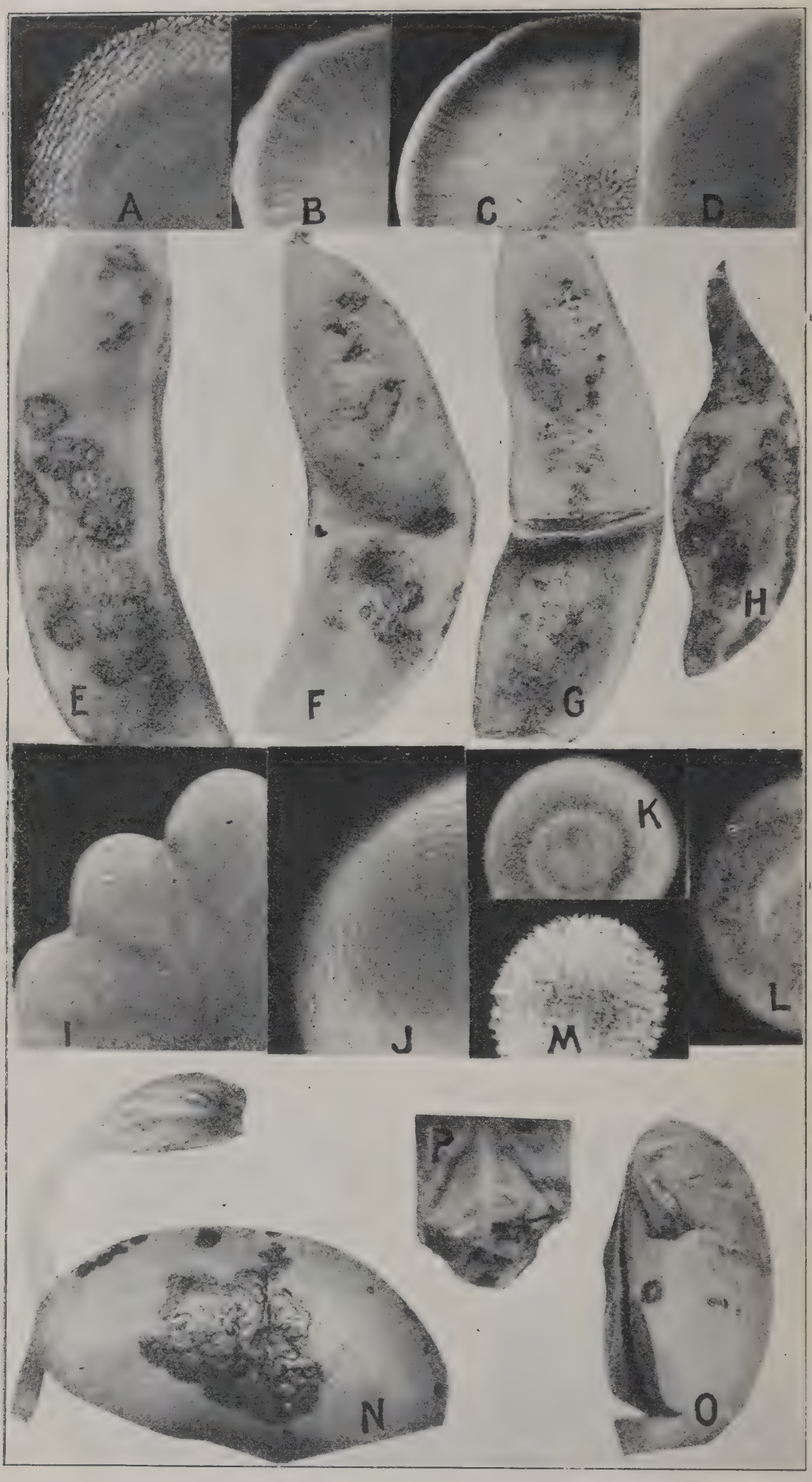
A colony of *Bact. phaseoli* EFS. produced infection on Lima bean pods 3 years 11½ months after its isolation. It was not, however, as virulent as colonies from more recent isolations.

EXPLANATORY LEGEND FOR PLATE 6

Bacterium phaseoli sojense. (Photographs by JAMES F. BREWER)

Colony types on beef agar plates, all pathogenic. ×10. All markings are internal, surface smooth.

A.—From Lima bean pod (Pl. 4, E), convoluted colony 6 days old.
B.—From pustule on soybean (Pl. 1, E), colony with radiating lines; colony 3 weeks old.
C.—From pale green spots with red centers on soybean; convoluted colony 9 days old.
D and E.—On same isolation plate, from soybean: E, concentric striæ; colonies 7 days old.
F.—From bush string bean (Pl. 4, A); colony 8 days old.
G.—From yellowish-green spot on dwarf wax bean; colony 5 days old.



Bacterial Pustule of Soybean

Plate 7

(For explanatory legend, see p. 249)

TRANSMISSION OF SOYBEAN PUSTULE

Very little is known regarding the transmission of the disease. Circumstantial evidence points to seed and perhaps insect transmission, although there is little experimental evidence.

SEED TRANSMISSION.—In the spring of 1919 some very badly diseased seed was received from Arkansas. Bacteria were found in some of the blackened spots, but all attempts at isolation failed, the plates being quickly overgrown by a spreading green fluorescent organism. Some of the seeds were then germinated and in 10 days 7 out of the 12 seedlings were badly diseased. The stems were water-soaked and full of bacteria, and 11 seedlings were broken over. *Bact. phaseoli sojense* was isolated and proved to be very infectious to Lima and bush string bean. There were many secondary infections also as new leaves unfolded on the diseased plants. No infection was obtained on Ito San soybean, however, although this is a very susceptible variety, and the plants inoculated ranged from seedlings 1½ inches high with no leaves to plants 8 or 9 inches high with leaves in all stages of development. Both spray and prick inoculations were made (inoculations of June 18 and 26, 1919).

INSECT TRANSMISSION.—Inasmuch as the disease was observed on 40 varieties of soybean growing in close proximity at Arlington Farm, Va., 1921, and raised from seed from widely separated regions,[18] it seemed reasonable to suppose that some of the seed at least was sound and that the plants became infected from their neighbors directly or indirectly. In the Dismal Swamp in 1917 the spotted cucumber beetle (*Diabrotica duodecimpunctata*) had been found on the diseased plants and a series of experiments was planned in an attempt to discover whether this insect might be a carrier of the infection. The results were negative. In the first experiments the beetles were fed on diseased leaves but they seemed to avoid the infected areas, which were rather hard and dry at this stage. Other beetles were fed on leaves smeared with *Bact. phaseoli sojense* and on potato cultures of it, which they seemed to relish, then transferred to healthy soybean plants. Although they fed on the leaves of the healthy plants, no infection resulted. It may be that temperature and moisture conditions were not favorable. The plants were in insect cages in the hothouse and no attempt was made to keep them in a moist atmosphere at any time during the experiments (carried on in October and November).

OTHER MEANS OF TRANSMISSION.—There is a possibility, of course, that the organism is spread by the rain and that it lives over on soybean refuse in the soil, but no observations have been made to support this theory.

SUMMARY

1. This paper describes a leafspot of soybean (*Glycine hispida*) produced by *Bact. phaseoli sojense* Hedges and characterized in the early stages by minute pustules and later by irregular reddish-brown spots, sometimes accompanied by yellowing.

2. *Bact. phaseoli sojense* is a short, 1-to 2-polar flagellate rod, very closely resembling *Bact. phaseoli* EFS. in most respects, but its colonies are commonly characterized by certain very striking internal markings, which are almost wholly absent from colonies of *Bact.*

[18] There were 177 different lots of seed, 124 of them having been received in 1921 and hence grown for the first time at Arlington. The seed had been obtained from Arkansas, Delaware, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, New York, Ohio, Pennsylvania, South Dakota, Tennessee, Virginia, West Virginia, Wisconsin, China, Java (some seed received from each of the foregoing localities in 1921), Japan, and Manchuria.

EXPLANATORY LEGEND FOR PLATE 7

Bacterium phaseoli EFS. (Photographs by JAMES F. BREWER)

Colonies on beef agar plates; inoculations on Lima bean pods and soybean seedlings.

A to D.—Colonies 10 days old. (×10, except C, which is ×5.) First two are in same poured plate and descended from a colony like type A.

E to H.—Lima bean pods six weeks after inoculation with colonies A to D, respectively. ×1.

I to M.—Rarer types, except J, which is frequent. (J is 6 days old.) L has depressed center. All except K and M, tested and found pathogenic. ×5–10.

N and O.—Germinating soybean seed sprayed in damp chamber with *Bact. phaseoli* EFS. Sown in damp chamber November 24, 1920, inoculated November 26, 1920, and photographed eight days later; water soaking and bacterial ooze on cotyledons; organism reisolated. ×3 ca.

P.—Browned and curled leaf tip from soybean seedling No. 27 grown from seed germinated in damp chamber (sown November 24, 1920), transferred to Sachs' solution December 1, sprayed in greenhouse December 2, with *Bact. phaseoli* EFS.; organism reisolated from similar leaves. Photographed eight days after inoculation. ×2.

phaseoli EFS. Furthermore, soybeans are not readily infected by *Bact. phaseoli* EFS.

3. *Bact. phaseoli sojense* does not produce pustules on Phaseolus. On the other hand, the best infections on the latter are not to be distinguished from those caused by *Bact. phaseoli* EFS.

4. The pustules are caused by both hypertrophy and hyperplasia chiefly of the parenchyma.

5. *Bact. phaseoli sojense* is easily isolated from fresh material by the poured-plate method. It has also been isolated from herbarium material 8 months old.

6. Artificial infection has been obtained by pure culture inoculation on a number of varieties of soybean, and natural infection has been observed on 40 varieties in experimental plats at Arlington Farm, Va.

7. Successful inoculations have been made on Lima, string, and wax beans of the genus Phaseolus.

8. There is some evidence that passage of *Bact. phaseoli sojense* through Phaseolus increases its virulence for the same.

9. In comparative inoculations on Phaseolus with *Bact. phaseoli sojense* (directly from soybean) and *Bact. phaseoli* EFS. the latter was much more infectious.

10. *Bact. phaseoli* EFS. is only very weakly pathogenic to soybean plants. Only once did infection occur in a long series of hothouse inoculation experiments made according to the method which was so successful with this organism on Phaseolus, or with *Bact. phaseoli sojense* on Phaseolus or soybean. In this exceptional case infection was very slight and did not resemble that caused by *Bact. phaseoli sojense* on soybean. Brown spots and streaks but *no pustules were produced.* The organism was reisolated.

11. *Bact. phaseoli* EFS. produced infection on soybean seedlings (1) grown from seed treated with formaldehyde or sulphuric acid and germinated in damp chamber and inoculated therein immediately after germination; (2) grown from formaldehyde-treated seed, germinated in damp chamber, transferred to Sachs' solution as soon as the seedlings had made sufficient growth, and inoculated eight days after sowing; (3) growing in pots from formaldehyde-treated seed germinated in damp chamber and sprayed in inoculating cages when they had developed the first pair of leaves, the leaves being rubbed between the thumb and forefinger after the spraying. No infection on leaves not so rubbed. (4) Grown from seed treated with sulphuric acid, washed, dipped in a water suspension of *Bact. phaseoli sojense* and germinated in damp chamber. By far the best infections were obtained in (1). *In none of these experiments were pustules formed.*

12. With the exception of the colonies on beef agar plates *Bact. phaseoli sojense* behaves on all the culture media tested like *Bact. phaseoli* EFS. Some additional cultural work was done with the two organisms. Some of the most favorable media are steamed potato cylinders, whey agar, potato agar plus 10 per cent dextrose, and Kellerman's synthetic agar plus Congo red.

13. Neither *Bact. phaseoli sojense* nor *Bact. phaseoli* EFS. loses its virulence readily, and both can be kept alive in beef agar stabs in the ice box for a year or more if small-bore (1½-cm.) tubes are used.

14. The disease is known to occur in Texas, Virginia, Louisiana, South Carolina, Kansas, Delaware, and Arkansas.

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VITAMIN A CONTENT OF FRESH EGGS[1]

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The significance of vitamins from the general standpoint of health and nutrition is now so well established that all new data on their content in foods are of prime importance. Several characteristic diseases and functional disorders are associated with a lack or deficiency of specific vitamins in the diet. Among these are beriberi, xerophthalmia, scurvy, rickets, impairment of the process of reproduction, failure of growth in young animals, and decline in weight in adults. The different vitamins are so distributed in our common food products that the diet of the average person probably contains an adequate supply. Nevertheless, through force of circumstances or on account of individual preference, many persons live on a diet so restricted that these food accessories are not obtained in sufficient quantities to meet nutritional requirements or to insure the optimum of health and well-being. This is particularly true in the case of small children. Cramer (*3*)[2] has recently pointed out that a borderland between health and disease may be created by a restriction in the vitamin supply. A diet may contain enough vitamins to afford protection from obvious ill health or interference with the breeding and rearing of the stock, and yet be so restricted as to lead to imperfect development and deviations from the optimum which up to the present have been overlooked.

It is becoming increasingly recognized that deficiency diseases and disorders caused by an insufficient intake of vitamins are of more frequent occurrence than has been generally supposed. Cramer (*3*) refers to outbreaks of an eye affection in England which have been ascribed to a lack of sufficient vitamin A and which were eliminated by the administration of cod liver oil. An account has also recently been given by Bloch (*1*) of a surprisingly large number of cases of xerophthalmia among children in Denmark who had not been getting sufficient vitamin A. The large number of cases of malnutrition among children in our public schools, and the beneficial effects produced by the recently introduced practice in many schools of furnishing milk, emphasize the need of greater attention to the qualitative and quantitative composition of the dietary.

It has been aptly stated that "vitamins should be obtained from the dairy, the grocery and the market, not from the drug store." Among the best known sources of vitamin A are cod-liver oil, butterfat, spinach, tomatoes, and egg yolk. From the standpoint of the health and nutrition of the general public the importance of any particular foodstuff as a source of vitamin must be considered from certain other angles as well as from its vitamin content. Of two substances having the same vitamin content, that one will be of the greater general importance which is the less expensive; which is more readily available to all people at all seasons and in all places; which combines with its vitamin value the greater food value with respect to other dietary factors; which is the more digestible, the more palatable, and better tolerated by infants and invalids. In accordance with these considerations, eggs must be considered a very important, if not the most important, source of vitamin A. Eggs and milk are the two articles which in themselves come nearest to being perfect foods. They contain all of the dietary factors essential for the nutrition of an animal during the early stages of its existence.

Hess (*4*) found that egg yolk is well tolerated by babies. He reported that for six months about 50 babies were fed with excellent results a mixture consisting of milk (24 oz.), barley water (12 oz.), sugar (¾ oz.), and 1 egg yolk; and he states that "egg yolk possesses marked antirachitic properties—far more than any other natural food product (*5*)." That egg yolk is a rich source of vitamin A was pointed out by Osborne and Mendel (*7*) and by McCollum and Davis (*6*) in their early work on this vitamin. Working with the yolk of fresh eggs, Hess found that 0.25 gm. fed daily was sufficient to protect rats from rickets. Casparis,

[1] Received for publication July 11, 1924; issued January, 1925. This paper was presented at the 67th meeting of the American Chemical Society held in Washington, D. C., Apr. 21 to 26, 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 257.

Shipley, and Cramer (*2*) obtained similar results. Hess states that a smaller quantity than 0.25 gm. of egg yolk would probably have been sufficient; but no attempt was made to force the feeding to a minimum.

EXPERIMENTAL

COMPOSITION AND PREPARATION OF DIET

The experiments described in this paper were undertaken to ascertain, as nearly as present methods permit, the smallest quantity of fresh whole egg which will cure rats of xerophthalmia, and enable them to grow at a normal rate.

The eggs used were laid during the summer months, and were from hens that had access to plenty of fresh, green food.

Young albino rats weighing from 45 to 55 gm. were placed on a basal ration found experimentally to be practically free from vitamin A, which had the following composition: Casein, 15 per cent; corn starch, 65 per cent; Crisco, 15 per cent; salt mixture, 5 per cent. Rats on this diet usually developed xerophthalmia in 40 to 50 days.

Vitamin B was supplied by 0.2 gm. of a commercial autolysed yeast preparation (marmite) fed separately each day.

The casein was prepared by precipitation at its isoelectric point from fresh skimmed milk, and purified by dissolving it in dilute sodium hydroxide and reprecipitating with acetic acid. This treatment was followed by extraction with alcohol and ether. The casein was then dried at 110° C. The starch was not specially treated, since it was not found to contain a detectable quantity of vitamin. Air was bubbled through the Crisco at about 100° for 48 hours, to insure the destruction of any vitamin which may have been present. The salt mixture used was that described by Osborne and Mendel (*8*). The egg was thoroughly beaten to a homogeneous consistency, and fed daily together with the marmite, separate from the rest of the diet.

POTENCIES OF VARIOUS QUANTITIES OF THE WHOLE FRESH EGG FOR THE PROMOTION OF GROWTH AND CURING OF XERPHTHALMIA

No curative dosage of egg was given until the rats had developed definite xerophthalmia. On a vitamin A free basal diet young rats usually make an initial growth at a fair rate for a few weeks and then cease to grow or begin to decline in weight. Many investigators begin to feed the material to be tested at this point, and note the effect on the growth of the animals as an indication of the vitamin potency of the material. Sometimes the first decline in weight is but temporary, and growth for a short period again follows, even without the addition of any vitamins. But waiting until xerophthalmia is evident before giving the substance to be tested may not only prevent a possible error due to this phenomenon, but permits a study of the effect of the vitamin A both as an antixerophthalmic and as a growth-promoting factor. These functions may be distinct and not strictly parallel.

The first trials were made with 0.5 gm. of egg given daily after the onset of xerophthalmia. The results are shown in Figure 1. As can be seen, the feeding of the egg promptly arrested the decline in weight, and enabled the rats to grow at a very fair, although not normal, rate. Xerophthalmia, which was very severe in the case of rats No. 1783 and No. 1785, was entirely cured in from 10 to 25 days and at the close of the experiment all the rats were in very good condition.

After it was found that 0.5 gm. of egg daily would cure xerophthalmia, without, however, permitting normal subsequent growth, several rats were fed 0.75 gm. of egg (fig. 2). The response to this was prompt. Rat No. 1794 developed an abnormality of tooth growth which interfered with feeding, and after an initial period of rapid gain had to be removed from the diet. Rat No. 1857, after having been given the egg, grew at a rate far exceeding normal. The eyes of all cleared up within a week.

It is evident that 0.75 gm. daily of fresh egg was sufficient to bring about normal growth in rats which had declined in weight and had developed xerophthalmia as a result of a lack of vitamin A in the diet. Growth at a rate of only about two-thirds of normal was secured on 0.5 gm. of egg. The minimum quantity of egg required to admit of normal growth can not, however, be safely decided from these experiments, since the rats were suffering badly from avitaminosis when the egg feeding was started. Consequently, more egg might have been necessary to permit subsequent growth at a normal rate than would have been the case had they received the egg from the beginning of the experiment. In another series of experiments in which 0.5 gm. of egg was fed daily from the beginning to young rats weighing about 50 gm., growth at the normal rate was secured for about 100 days, after which the growth became subnormal. From

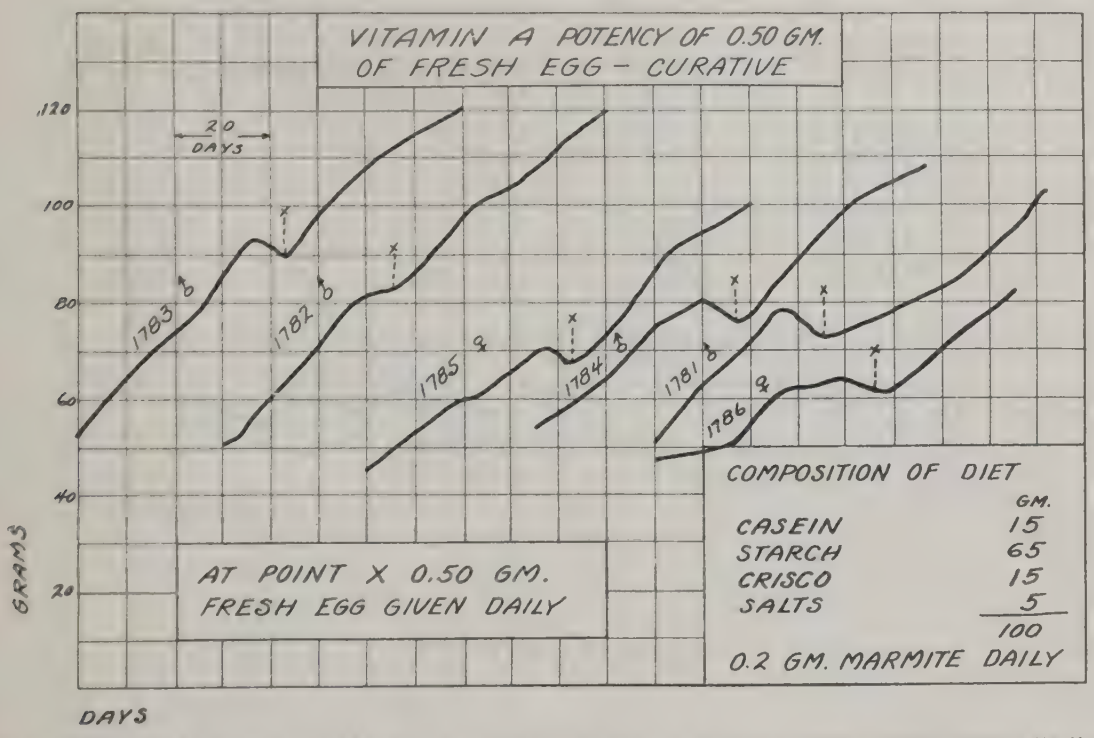

FIG. 1.—Vitamin A potency of 0.50 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

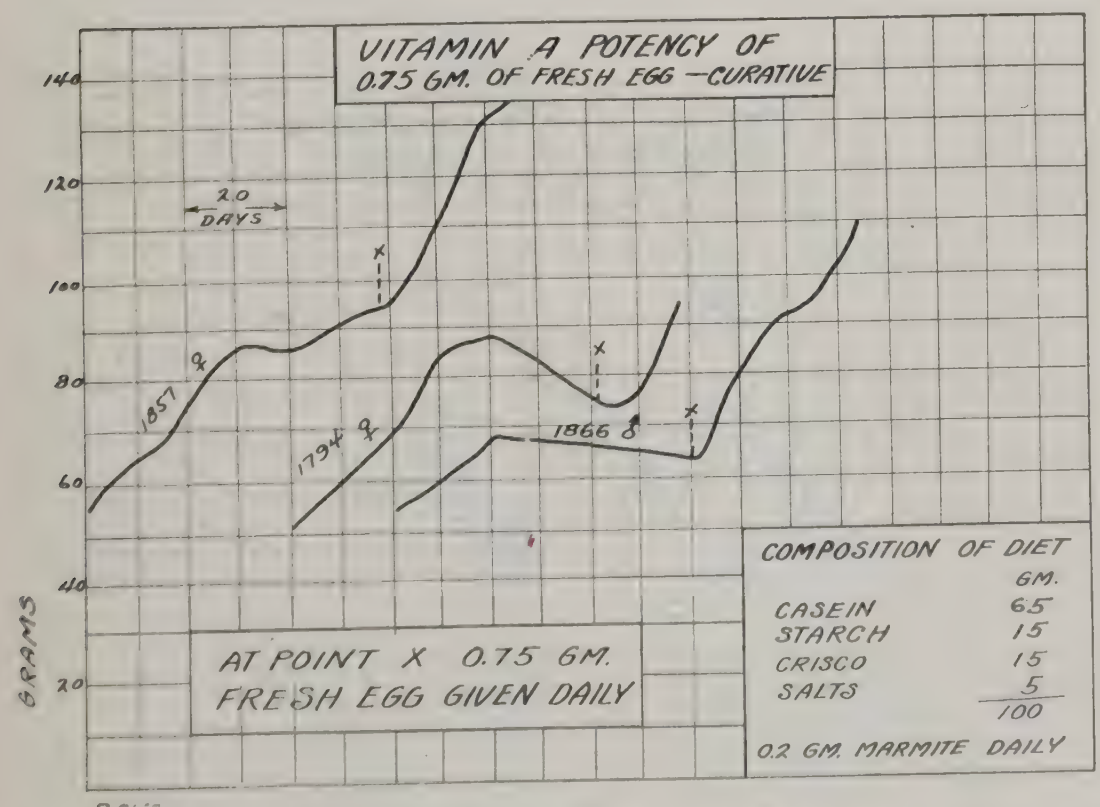

FIG. 2.—Vitamin A potency of 0.75 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

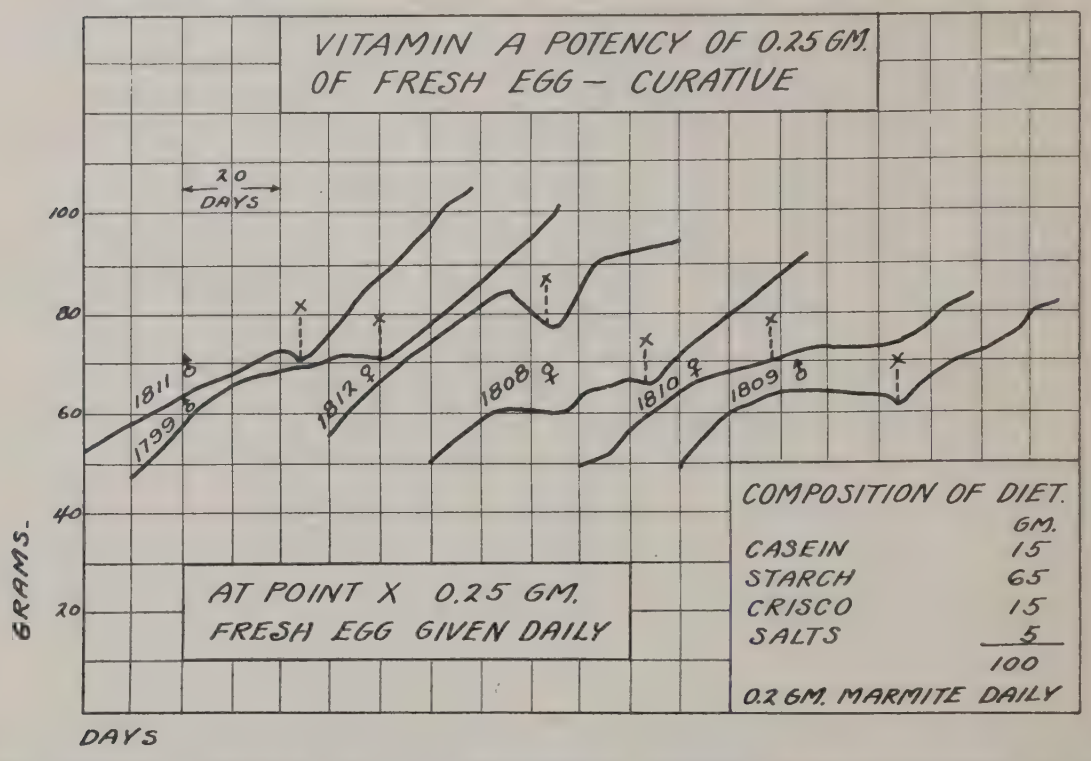

FIG. 3.—Vitamin A potency of 0.25 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

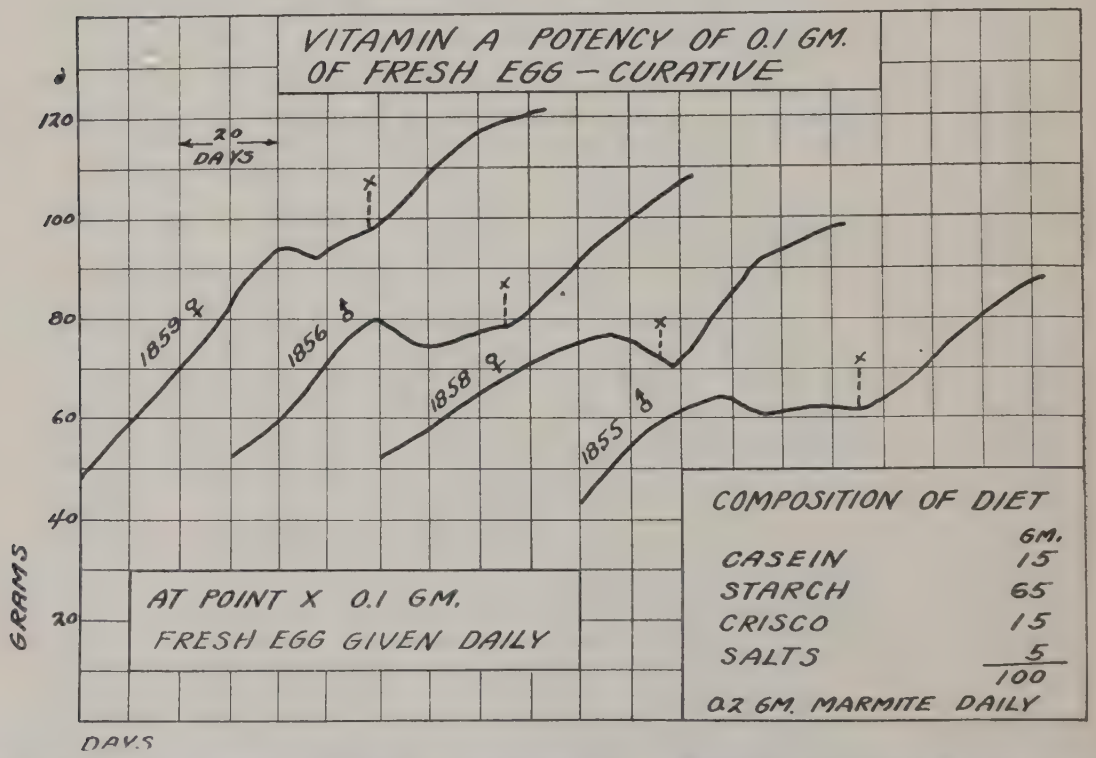

FIG. 4.—Vitamin A potency of 0.1 gm. of whole fresh egg. Beginning at the points marked "x," the curative dose of egg was given daily after the rats had shown unmistakable symptoms of xerophthalmia

these results it would appear that the minimum quantity of vitamin A required for the normal growth of rats is furnished by an amount of fresh whole egg very little more than 0.5 gm.

Since 0.5 gm. of egg was found ample to cure xerophthalmia, smaller amounts were tried, to determine the minimum dosage that could be used. Accordingly, six rats were fed 0.25 gm. of egg. The results in this case were decidedly poorer than those with the 0.5 gm. dose (fig. 3). Growth was slightly less, and the length of time required to cure the sore eyes was very much increased. One rat, No. 1808 while showing fair growth, still exhibited distinct signs of sore eyes at the close of the experiment, 38 days after the beginning of the egg feeding.

The lower limit seemed to have been reached at 0.25 gm., but another series was tested with 0.1 gm. of egg (fig. 4). Although the growth recovery of these was not far different from that of those receiving 0.25 gm., the effectiveness of 0.1 gm. for curing xerophthalmia was found to be very slight. Only one rat, No. 1856, was completely cured after three weeks of feeding; and the others, even after six weeks, gave no indication that they would ever recover fully on this dosage.

The fact that the weight increase of the rats receiving 0.1 gm. of egg was nearly identical with that of those receiving 0 25 gm., and not far below that of those receiving even 0.5 gm., is a somewhat surprising phenomenon which is being studied by the authors.

DISCUSSION

These experiments indicate that test rats, after having declined in weight and having developed xerophthalmia as a result of vitamin A deficiency, can be restored to normal weight by feeding 0.5 to 0.75 gm. of whole egg daily; and that 0.5 gm. is very near the quantity required to enable healthy young rats to maintain growth at a normal rate. If weight increase be not considered, but only the effectiveness for curing xerophthalmia, the lower limit is reached at about 0.25 gm. Only one rat failed of complete cure on this dosage, and this one might possibly have recovered eventually.

It is of interest to calculate these figures in terms which are sometimes used by other workers. Since the yolks compose about 35 per cent of the whole egg, 0.75 gm., 0.50 gm. and 0.25 gm. of whole egg would then be equivalent to 0.26 gm., 0.17 gm., and 0.088 gm. respectively, of yolk. The minimum quantity (0.25 gm. of yolk) which Hess found sufficient to protect rats from rickets was, therefore, very close to the minimal growth-restoring dose. Of the whole yolk 49.8 per cent is water. The quantity of egg yolk required, calculated to a dry basis, would therefore be between 0.083 gm. and 0.13 gm. for growth restoration, and 0.041 gm. for the cure of xerophthalmia. On the assumption that all of the vitamin A is contained in the oil of the yolks, then, since 60 per cent of the dried yolk is oil, a little more than 0.05 gm. of egg oil would be required for growth promotion, and 0.025 gm. would be required for the curing of xerophthalmia. Cod-liver oil has been found effective in doses of about 0.001 gm. Therefore egg oil would have a vitamin A potency between 2 and 4 per cent of that of the most potent cod-liver oil.

SUMMARY

Feeding experiments have shown that from 0.50 gm. to 0.75 gm. of fresh, whole egg fed daily supplies young rats with sufficient vitamin A for growth at a normal rate. A smaller quantity, 0.25 gm, was found to be adequate to cure well advanced cases of xerophthalmia.

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SOME INSECTICIDAL PROPERTIES OF THE FATTY ACID SERIES [1]

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The use of soaps as contact insecticides is of long standing in economic entomological practice, although there has apparently been no serious investigation leading toward the determination of the active principle chemically responsible for their value as such. References available to the writers attribute the toxicity of soaps to their alkaline ingredients, expressing the belief that their other constituents, the fatty acids, are practically inert in their action toward insects. In the belief that the results obtained through an investigation of this problem during the past year justify their presentation for the consideration of other investigators, this brief preliminary paper, including some of the theoretical aspects and a promising practical application to insecticide entomology, is herewith presented.

Soaps have been defined as the alkali salts of the fatty acids. Since those most commonly in use are formed from the fatty acids containing an even number of carbon atoms united in a straight chain, or the normal, saturated monocarboxylic fatty acids found in nature, this series was chosen as the subject of the tests first conducted, as herein outlined. A complete series of the even-carbon acids through stearic acid was tested, several of the odd-carbon acids being likewise compared. When it was possible to obtain them, purified acids were used, although the technical grades were employed when necessary. The following homologues of the series $C_NH_{2N}O_2$ were available: Acetic, propionic, butyric, valeric, caproic, oenanthylic, caprylic, pelargonic, capric, lauric, myristic, palmitic, and stearic acids.

The results obtained on the available species of aphids were striking. While no great toxicity has been encountered with the lower homologues, the caproic acid showed marked toxicity, and tests of the next in the series, caprylic acid, were gratifying. Kills of more than 90 per cent of the black chrysanthemum aphid were attained at 1 to 500 dilutions, with only slightly decreased results when the water proportion was increased to 1 to 1,000. Capric acid, the next even-chain homologue, killed more than 99 per cent of the green apple aphid at a dilution of 1 part to 1,200 of water. Lauric acid at the same dilution was fatal to 92 per cent of the same aphid, while myristic acid at 1 to 1,200 killed 78 per cent of the same species.

Paralysis is complete and practically immediate when aphids are subjected to toxic strengths of the fatty acids. It was noted in tests on the black chrysanthemum aphid in comparison with nicotine sulphate (commercial 40 per cent) that in the case of the latter poison the mortality was indicated by the large percentage of dead aphids which dropped from the sprayed plants. In the case of the fatty acids, the dead insects remained attached by their inserted beaks, affording no gauge of toxicity, such as was shown by the nicotine, until the actual counts were made.

The fatty acids tested were applied in the free form, and as acid, neutral, and alkaline soaps, using potassium, sodium, and ammonium bases. In the free form, they were emulsified by means of various stabilizers, and when used with distilled water alone, by means of a colloid mill. In the later part of the investigation a solution of glue was used as a standard emulsifier, providing ample stability for uniform experimental tests. Throughout the study parallel tests, using a commercial 40 per cent nicotine sulphate solution in combination with soap, were conducted for comparison, and ample check or untreated material was kept under observation.

Practically all tests were made on a laboratory scale in an outdoor insectary. The insects were thoroughly wetted by means of a small atomizer drawing from the bottom of the chamber.

It is hoped that at a somewhat later date detailed information as to the results of the season's work may be presented.

[1] Received for publication Sept. 12, 1924—issued January, 1925.

The principal subjects of the tests have been the rosy apple aphid, *Anuraphis roseus* Baker, the green apple aphid, *Aphis pomi* DeGeer, the bean aphid, *Aphis rumicis* Linn., the black chrysanthemum aphid, *Anuraphis sanborni* Gill., the black cherry aphid, *Myzus cerasi* Fab., and the aster aphid, *Macrosiphum ambrosiae* Thos. Preliminary tests further indicate that this material is toxic to insects of other orders as well as to Acarina.

The physical characteristics of the fatty acids tested lend themselves admirably to use as contact insecticides. The fatty acids showed unusual wetting and spreading powers when applied in the emulsion form to either insects or foliage, wetting readily such insects as the polished black aphids, the squash bug, and hairy caterpillars. Leaves having a waxy covering, such as nasturtium and cabbage, are easily and evenly coated. The physiological action of the fatty acids on the insect organism is as yet undetermined.

Experiments so far conducted indicate that the toxicity of the fatty acids increases with the molecular weight, at least to a certain point not yet definitely determined. A practical amount of toxicity was reached with the sixth carbon atom, the peak of toxicity apparently lying at or slightly above the C_{10} point. Toxicity is much greater in acid mixtures, the free acids killing at materially higher dilutions than when combined as their corresponding soluble alkaline or neutral salts. In contradistinction to the action of nicotine, no variation in toxicity is experienced under varying summer temperatures and humidity, leading to the conclusion that the low volatility of the fatty acids insures their retention in the body of the insect until action is complete.

Plants show a varying susceptibility to burning by the acids of this chemical series. The foliage of the apple is not affected when sprayed with strengths toxic to apple aphids. No injury to chrysanthemum plants was shown by dilutions fatal to the black chrysanthemum aphid. On the other hand, the foliage of nasturtium is injured by the dilutions required to kill the bean aphid.

Tests toward the practical application of the insecticidal properties shown by preliminary work with the long-chain fatty acids resulted in the selection of a product commercially known as "double distilled coconut fatty acids" as embodying in the greatest degree the desirable features of those members of the series approximating the peak of practical toxicity. A typical sample of this product, much used industrially in soap manufacture and otherwise, was fractionated [2] with the following results:

	Per cent.
A.—Fraction mostly caprylic acid (small amounts caproic and capric)	4. 0
B.—Fraction mostly capric acid (small amounts caprylic and lauric)	14. 6
C.—Fraction mostly lauric acid (small amounts capric and myristic)	51. 1
D.—Fraction mostly myristic acid (small amounts lauric and palmitic)	14. 1
E.—Fraction mostly palmitic acid (small amounts myristic, oleic, and stearic)	8. 3
F.—Fraction mostly oleic and stearic acids (small amount palmitic)	5. 6
	97. 7

The fractions A to D as shown were individually tested for contact toxicity, fraction B, containing mostly capric acid, showing slightly greater toxicity than either A, C, or D. This tends to confirm the theory that the peak of toxicity lies near capric acid $C_9H_{19}COOH$.

The commercial product selected is liquid at usual summer temperatures (M. P. 27° C.), highly toxic even when greatly diluted, stable with lubricating-oil emulsions, readily obtainable in quantity, and at the time of writing is much lower in price than nicotine. Difficulties in the preparation of a stock solution and the retention of the insoluble acids in a stable emulsion have been met by the addition of an equal amount of benzol gasoline to the commercial fatty acids, afterwards using powdered glue as a colloidal stabilizer. The gasoline lowers the melting point to 5° C., thus serving to prevent solidification of the acid globules in the emulsion through lowered temperatures, also facilitating even distribution, while the glue greatly retards separation after emulsification. The stock emulsion [3] has been tentatively

[2] This work was done by the Insecticide Laboratory of the Bureau of Chemistry, United States Department of Agriculture.

[3] *Stock solution*

Coconut fatty acids (double distilled)	200 cc.
Gasoline (benzol)	200 cc.
Glue (granular)	100 gm.
Water	525 cc.

adopted as a practicable basis for further tests. In series of tests with this formula, used in the proportions of 1 part of the coconut fatty acids to from 800 to 1,200 parts of water, a mortality rate has been attained against the green apple aphid of from 94 to 98 per cent, equivalent to the rates obtained with commercial 40 per cent nicotine sulphate in parallel tests at the same dilutions.

Approximately a liter of stock solution is obtained when made up as indicated, each 5 cc. containing 1 cc. of the commercial mixture of acids. Upon thorough shaking it readily becomes a milky emulsion, capable of dilution with any ordinary proportion of water, and when so diluted it remains sufficiently stable for practical application. The spreading power of the emulsion is excellent, toxicity high, and cost of material extremely low. Prepared by this formula, coconut fatty acids have compared favorably in efficiency, pound for pound, in the experiments so far conducted, with commercial nicotine preparations at less than one-fourth the cost per gallon of spray mixture.

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INFECTION OF BARLEY BY USTILAGO NUDA THROUGH SEED INOCULATION [1]

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INTRODUCTION

Floral infection of barley (*Hordeum sativum* L.) by *Ustilago nuda* (Jens.) Kell. and Sw., as well as floral infection of wheat (*Triticum vulgare* Vill.) by *Ustilago tritici* (Pers.) Jens., has been considered an established fact for many years. There was considerable confusion regarding the infection of cereals by smut fungi before the different species of these fungi were identified and described. Since these differences were found steady progress has been made in the discovery of the morphological and physiological differences of the smut fungi, of their host relationships, and of the control of the smuts caused by them.

Since the discovery of floral infection (*16*)[2] the hot-water seed treatment has been considered the only means of controlling the loose smuts of wheat and barley. Recently, however, formaldehyde and some of the organic mercury compounds have been found to control the loose-smut of barley in certain varieties (*13*, *20*, *21*, and *22*). This led to the belief that there remained important facts to be learned regarding the infection of barley by *Usitlago nuda*.

In the autumn of 1922 the senior writer planned to study the possibility of seedling infection by this fungus. The results of the first experiment were so striking that a number of experiments, in which nine varieties of barley were used, was conducted during the past season, 1923–24. The results of these studies were even more striking than those of the previous year.

This paper gives a brief review of the literature dealing with floral infection of wheat and barley by their respective loose-smut fungi, presents the data of the writers' recent studies showing that previous investigators have been confused regarding the infection of barley by *Ustilago nuda*, at least to a certain extent, and that the fungus infects the seedlings, at least of certain varieties of barley, and causes severe injury to the seedlings when heavily infected.

HISTORY OF FLORAL INFECTION

Hoffman (*10*), in 1866, long before the discovery of the difference in the fungi causing the smuts of barley, mentioned the possibility of floral infection. He was studying barley smut principally but did some work with the smuts of wheat and oats.

In 1888, Jensen (*12*), after several years of study of infection of barley and oats by smut (*Ustilago segetum*), made the following statement:

> Since we have seen that spores adhering to the exterior of the grain do not to any appreciable extent cause the infection of the crop, it follows that this must take place by means of those spores which succeed in entering the space between the "cosh" or husk and the kernel.

He then suggested two possibilities for infection; one being floral infection by direct germination of the spores immediately after they enter the flowers, and the other being the infection of the seedlings by spores which remained quiescent between the husk and the kernel until the seed was sown and then germinated. He says that his experiments of 1887 point to the latter. He found that by removing the hulls (glumes) of barley and oats and inoculating the seed, higher percentages of infection could be obtained.

After a large part of these experiments was concluded Jensen found that there were two smuts of barley and described them as *Ustilago segetum*, variety *hordei nuda*, "the naked smut," and *Ustilago segetum*, variety *hordei tecta*, "the covered smut." Previous to this, he had assumed that the two were identical, *U. segetum* DC. After this discovery he says:

> In the experiment quoted above with barley smut the variety *tecta* only was used for infecting the bare kernels.

At about the same time, 1888, Brefeld (*1*) failed to obtain infection of barley by inoculating the seed and young seedlings with germinated and ungerminated spores. He then obtained smutty barley heads from Japan and found that the spores from these

[1] Received for publication Aug. 23, 1924. Issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 283–284.

heads germinated and formed mycelium but no conidia. Conidia were produced by the form previously used. He called the form from Japan *Ustilago hordei*. This was evidently the form which is now known as *U. nuda* which causes loose-smut. Brefeld evidently made his infection studies with the fungus of covered smut, *Ustilago hordei*. He makes no mention in this paper of further infection studies after discovering this difference in the barley smut fungi.

In 1899 Kellerman and Swingle (*14*), in a study of the barley smuts, described the two species *U. hordei* (Pers.) Kell. and Sw., covered smut, and *U. nuda* (Jens.) Kell. and Sw., loose-smut. They cited Jensen's statements concerning floral infection but said, "This view requires confirmation."

Maddox (*16*) concluded from carefully conducted experiments that the loose smuts of wheat and barley could not be produced by smutting the seed or by mixing spores with the soil. He says:

> Artificially smutted is putting the smut germs on the ovary about the time the pollen is ripe, which will always reproduce the disease the following year.

According to Hori (*11*), K. Yamada in 1896 and S. Nakagawa in 1897 produced loose-smut in wheat by inoculating the flowers with mature spores of *Ustilago tritici*.

Brefeld (*2*) in 1903 discussed methods of floral inoculation of wheat and barley with the loose-smut spores. He concluded that infection takes place through the flower. Again in 1905 Brefeld (*3*) published, in detail, methods used in attempting to infect barley with *Ustilago nuda* by inoculating the flowers and seed with spores and by inoculating the young seedlings with germinated spores. In one experiment he obtained 1 per cent infection where the seedlings were inoculated with germinating spores, while two-rowed barley treated similarly remained smut free, as did the control. This was the only indication of seedling infection. Brefeld's results led him to conclude, however, that—

> infection in the blossom is the predominant form of infection of the host plants, if not the only one.

Hecke (*8*) produced floral infection in barley in 1904, and in 1905 he (*9*) figures the mycelium in the scutellum of barley. He adheres to Brefeld's terminology and calls the loose-smut fungus *Ustilago hordei*.

Hori (*11*) in 1907 states that he obtained floral infection by *Ustilago tritici* and *U. nuda* as early as 1900. After discussing the findings of Brefeld and Hecke he makes the following statement:

> Hence it is now clear that at least *U. tritici*, *U. nuda* and *U. hordei* may naturally infest the respective host plants by the flower infection.

Hori evidently was referring to the loose-smut of barley both as *U. nuda* and *U. hordei*. Brefeld (*1*) called the loose-smut fungus *U. hordei* when he first discovered that there were two barley smuts. Hori was probably confused by this difference in nomenclature.

Falck (*5*) in 1908 and Lang (*15*) in the following year studied the infection of wheat by *Ustilago tritici* cytologically and showed the mycelium in the stigma and the young ovaries and showed it ramifying in the cells of the embryo at various stages of development.

Freeman and Johnson (*7*) in 1909 studied the loose smuts of wheat and barley. They inoculated the flowers at different stages of maturity and found that infection takes place from the time when the stamens are still green to the time when the ovary is one-third its mature size. They found the optimum time for infection to be when the flower is in full bloom or when the ovary is just commencing to develop after fertilization.

Brioli (*4*) in 1910 studied floral infection in wheat and barley and confirmed the work of previous investigators. He illustrates the mycelium in the scutellum of wheat and says that he found mycelium in the scutellum of one variety of barley but not in the other varieties:

> Nur bei Körnern der Niederbayerischen Gerste habe ich Mycel gefunden. Bei den anderen Gersten nicht.

He attributes the lack of mycelium in the kernels to resistance of the host.

PRESENT INVESTIGATIONS

SEEDLING INFECTION

For many years after the discovery of the hot-water seed treatment by Jensen (*12*) this method was recommended as being the only treatment that would control the loose-smuts of wheat and barley. In 1914, Johnson (*13*) reported the control of loose-smut of barley by treating the seed with formalin. Tisdale, Taylor, and Griffiths (*21*) in 1923, obtained satisfactory control of loose-smut of barley by treating the seed with formaldehyde and with chlorophol, an organic mercury compound. Six varieties of barley were included in this experiment. Since that time Semesan, Corona 620,

Uspulun and Germisan (organic mercury compounds) have been found to control the loose-smut of barley (*20, 22*).

These results were surprising in view of the claims and apparent proof of floral infection of barley by *Ustilago nuda*. At the same time, it led to the belief that it might be possible that previous investigators were misled, in some way, regarding infection of barley by the loose-smut fungus. Of course, it seemed entirely possible that floral infection might take place and the fungus mycelium remain in the superficial cells of the kernel where it could be destroyed by disinfectants which penetrate the seed coat.

Tisdale (*19*) found that by inoculating dehulled[3] barley kernels with spores of the covered smut fungus *Ustilago hordei*, high percentages of infection could be obtained, while often little or no infection could be secured by inoculating the hulled seed. In the fall of 1922, while conducting the experiments on covered smut, the senior writer inoculated some dehulled seed of Tennessee Winter and Nakano Wase barleys with spores of *U. nuda* and sowed it on a bench in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. Dehulled-uninoculated, hulled-inoculated, and hulled-uninoculated seed of both varieties also was sown. The seed for this and subsequent experiments was dehulled by carefully removing the glumes with a sharp-pointed knife or scalpel. The basal end was first broken and the glumes stripped off. Nakano Wase remained smut-free in all experiments. The dehulled-inoculated seed of Tennessee Winter produced 25 per cent of loose-smut, or 5 plants in a total of 20, while only 1 plant in 22 from the dehulled-uninoculated and 1 in 20 from the hulled-inoculated seed produced loose-smut. (Table I.)

The occurrence of 25 per cent of loose-smut in plants from inoculated-dehulled seed appeared to be significant. This led to plans for further experiments in which both floral and seedling infection could be studied. Six varieties of barley were used in these studies. Floral inoculations were made in the spring of 1923 on barleys growing in the greenhouse. Each flower was carefully inoculated by opening the glumes with forceps and dusting the floral parts with fresh spores of *Ustilago nuda* from Tennessee Winter barley growing in the greenhouse. Flowers and seeds were inoculated in different stages and treated in different ways, as follows:

1.—Inoculated when anthers were green; no pollen shed.

2.—Inoculated when anthers were shedding pollen.

3.—Inoculated when pollen was recently shed, stigma plumose, and showing no signs of withering.

4.—Inoculation at tip of kernel when in the milk stage. The glumes were pulled back slightly, spores placed on the kernel, and the glumes replaced.

5.—Mature seed dehulled and sown without inoculation.

6.—Mature seed dehulled and inoculated by shaking thoroughly in an envelope with spores until almost black.

7.—Mature hulled seed sown without inoculation.

8.—Mature hulled seed inoculated.

Spore material used for inoculating the mature seed was collected from Tennessee Winter barley grown on Arlington Experiment Farm in 1923. The seed inoculated by the various methods given above was sown on a bench in the greenhouse on November 4, 1923. The seeds were sown about 1 inch deep and 2 inches apart in rows 7 inches apart. The results of these experiments are given in Table II.

TABLE I.—*Infection of barley seedlings by Ustilago nuda in the greenhouse in 1922–23*

Variety	Treatment	Number of plants		Percentage of smut
		Total	Smutted	
Tennessee Winter	Hulled-uninoculated	25	0	0
Do	Hulled-inoculated	20	1	5.00
Do	Dehulled-uninoculated	22	1	4.55
Do	Dehulled-inoculated	20	5	25.00
Nakano Wase	Hulled-uninoculated	21	0	0
Do	Hulled-inoculated	20	0	0
Do	Dehulled uninoculated	24	0	0
Do	Dehulled-inoculated	19	0	0

[3] Hulled = hulls not removed, and dehulled = hulls removed.

TABLE II.—*Infection of barley by Ustilago nuda in the greenhouse in 1923–24, by flower and seed inoculation* [a]

Variety	Type and stage of inoculation	Number of plants			Number of heads		
		Total	Smutted	Per cent of smut	Total	Smutted	Per cent of smut
Alaska	3. Pollen shed	81	3	3.70	233	13	5.58
Do	4. Milk stage	85	2	2.35	281	4	1.42
Do	5. Dehulled, uninoculated	15	0	0	41	0	0
Do	6. Dehulled, inoculated	47	36	76.60	188	148	78.72
Do	7. Hulled, uninoculated	30	0	0	115	0	0
Do	8. Hulled, inoculated	42	6	14.29	144	12	8.33
Greece	3. Pollen shed	64	1	1.56	210	1	0.48
Do	4. Milk stage	19	1	5.26	84	9	10.71
Do	5. Dehulled, uninoculated	29	0	0	101	0	0
Do	6. Dehulled, inoculated	49	31	63.27	142	80	56.34
Do	7. Hulled, uninoculated	23	0	0	69	0	0
Do	8. Hulled, inoculated	42	0	0	124	0	0
Han River	3. Pollen shed	5	0	0	14	0	0
Do	4. Milk stage	(b)	(b)	(b)	123	4	3.25
Do	5. Dehulled, uninoculated	11	0	0	44	0	0
Do	6. Dehulled, inoculated	33	29	87.88	146	132	90.41
Do	7. Hulled, uninoculated	33	2	6.06	110	3	2.73
Do	8. Hulled, inoculated	46	9	19.57	127	25	19.69
Nakano Wase	1–3. Anthers green to pollen shed	104	0	0	375	0	0
Do	4. Milk stage	65	0	0	284	0	0
Do	5. Dehulled, uninoculated	17	0	0	73	0	0
Do	6. Dehulled, inoculated	46	0	0	167	0	0
Do	7. Hulled, uninoculated	32	0	0	110	0	0
Do	8. Hulled, inoculated	35	0	0	127	0	0
Texas Winter	2–3. Pollen shedding to shed	72	5	6.94	187	19	10.16
Do	4. Milk stage	54	9	16.67	210	42	20.00
Do	5. Dehulled, uninoculated	9	1	11.11	21	3	14.29
Do	6. Dehulled, inoculated	14	14	100.00	114	114	100.00
Do	7. Hulled, uninoculated	35	0	0	154	0	0
Do	8. Hulled, inoculated	37	6	16.22	133	15	11.28
Wisconsin Winter	2–3. Pollen shedding to shed	79	4	5.06	274	9	3.28
Do	4. Milk stage	42	0	0	151	0	0
Do	5. Dehulled, uninoculated	26	0	0	89	0	0
Do	6. Dehulled, inoculated	40	21	52.50	143	73	51.05
Do	7. Hulled, uninoculated	21	0	0	75	0	0
Do	8. Hulled, inoculated	39	6	15.38	160	24	15.00

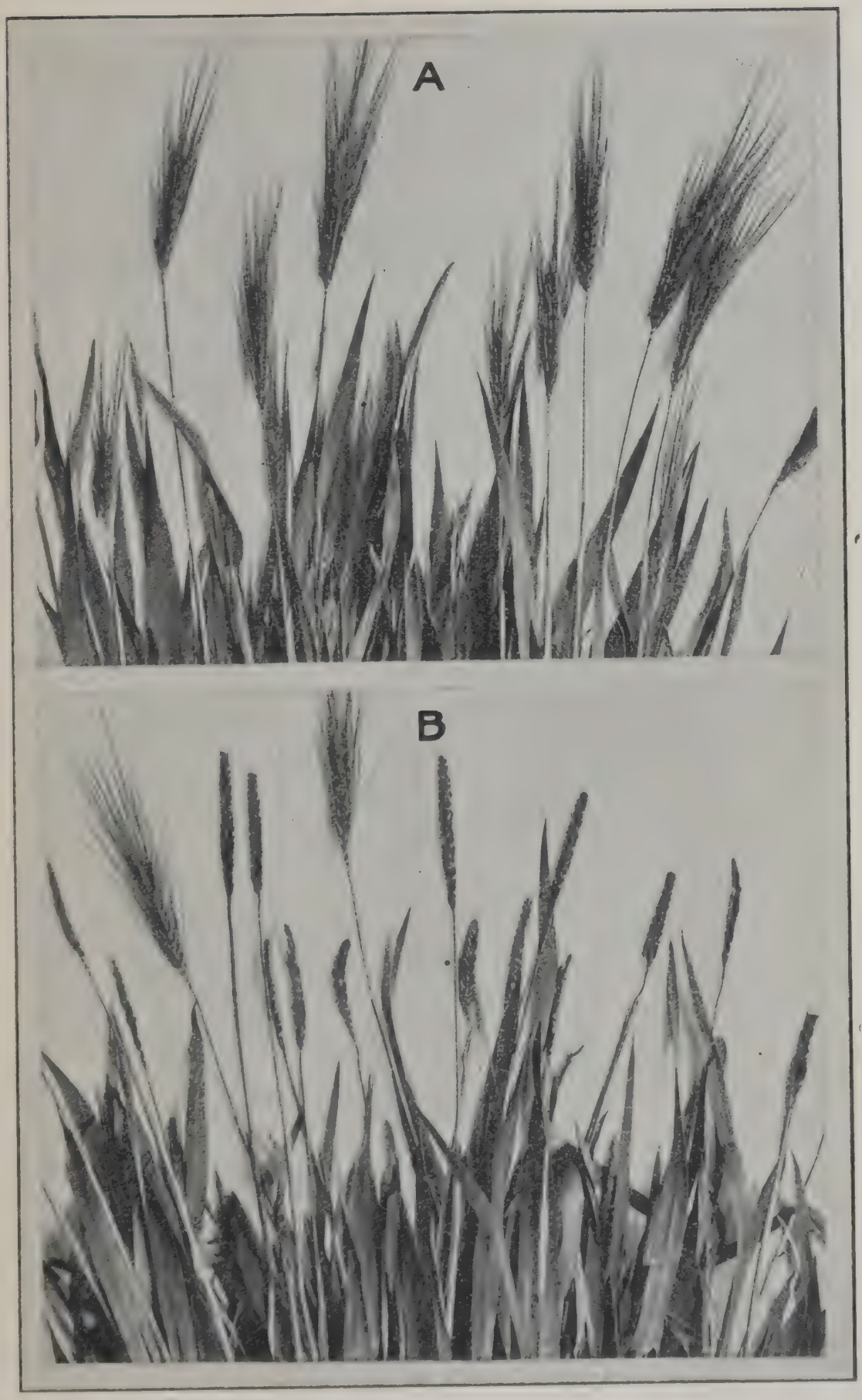
[a] The type of inoculation is referred to by number in the second column from the left in this table. The numbers refer to the explanations of time and method of inoculation given above.
[b] Not counted.

The smut records in Table II show strikingly that seedling infection takes place when the mature seed is dehulled and inoculated with spores of *Ustilago nuda* (Pl. 1, B). There evidently was a small amount of natural infestation in the seed used as two smutted plants occurred in one of the Han River controls and one plant in one of the Texas Winter controls. Other controls were smut-free (Pl. 1, A). The seed was not disinfected in any case as there was a possibility of injuring the vitality and interfering with the results.

Apparently some infection was obtained in some cases by dusting the spores on the stigma and ovary at flowering time and afterward. This appears to be true for all varieties except Han River and Nakano Wase, The percentages of infection, however, are not as high as have been obtained by previous investigators using this method of inoculation. As suggested by Jensen (*12*), there is a possibility that the flowers were infected, and also a possibility that the spores remained between the glumes and the kernel and germinated and produced seedling infection when the seed germinated. Microscopic examination three days after the flowers were inoculated showed that many of the spores had germinated within the flowers of Alaska barley. No infections were found. This limited examination does not prove, by any means, that infection does not take place. Neither does it prove that the ungerminated spores are not finally responsible for infection. Even though infection takes place in the flower, which seems entirely pos-

EXPLANATORY LEGEND FOR PLATE 1

Infection of Han River barley by *Ustilago nuda*. Plants grown on bench in greenhouse.
A.—Seed dehulled and uninoculated. All plants remained smut-free
B.—Seed dehulled and inoculated with spores of *U. nuda*; 87.88 per cent of the plants and 90.41 per cent of the heads were smutted.



Infection of Barley by Ustilago nuda

Plate 1

(For explanatory legend see p. 266)

sible, the mycelium within the seed may not be responsible for the infection which takes place when the seed is sown. The fact that loose-smut is controlled by superficial disinfectants would indicate that it is not internal unless it be very superficial so that these fungicides destroy it.

The results show that, to a large extent, the hulls (glumes) are the limiting factor in the infection of barley by *Ustilago nuda*. However, with the exception of Nakano Wase and Greece, comparatively high percentages of loose-smut were obtained by inoculating hulled seed, the lowest percentages of infected plants in the other four varieties being 14.29 per cent in Alaska. This amount of infection is sufficient to account for the percentages of loose-smut in barley which generally occur in nature. As is true with the covered-smut fungus, *U. hordei*, the inoculation of hulled seed with *U. nuda* does not insure infection. The hulls evidently are an important inhibitor. There may be different strains of the fungus, as Faris (*6*) has pointed out in the case of *U. hordei*, which infect certain varieties more readily. While there is a chance for the spores to enter between the glumes at flowering time when spore material is abundant, there seems to be an equally good or better chance for them to enter when the grain is being threshed and handled. The hulls often are broken and some kernels are completely dehulled in the threshing process. It is true that the loose-smut is largely dissipated by harvest time, but there no doubt is enough spore material left on diseased heads and surrounding plants to cause the low percentages of infection which ordinarily occur in nature. If barley is inoculated naturally by *U. nuda* in the same way as it is by *U. hordei* the lower percentages of loose-smut which generally occur would harmonize with the fact that the spores disappear more readily in the case of loose-smut and that there is much less inoculum available at threshing time

Nakano Wase remained smut-free in these experiments. It failed to become smutted with covered smut in the experiments of Tisdale (*19*). So far as the writers know, neither loose nor covered smut has been found in this variety in the field.

SEEDLING INJURY

In previous experiments it was noticed that in most cases the stands of seedlings from dehulled-inoculated seeds were poor as compared with the stands from dehulled and uninoculated seed. It even was necessary in some cases to reseed in order to get a satisfactory stand from inoculated seed. Many of the seedlings that emerged from inoculated seed were abnormal in appearance. Many of them emerged at an angle rather than normally.

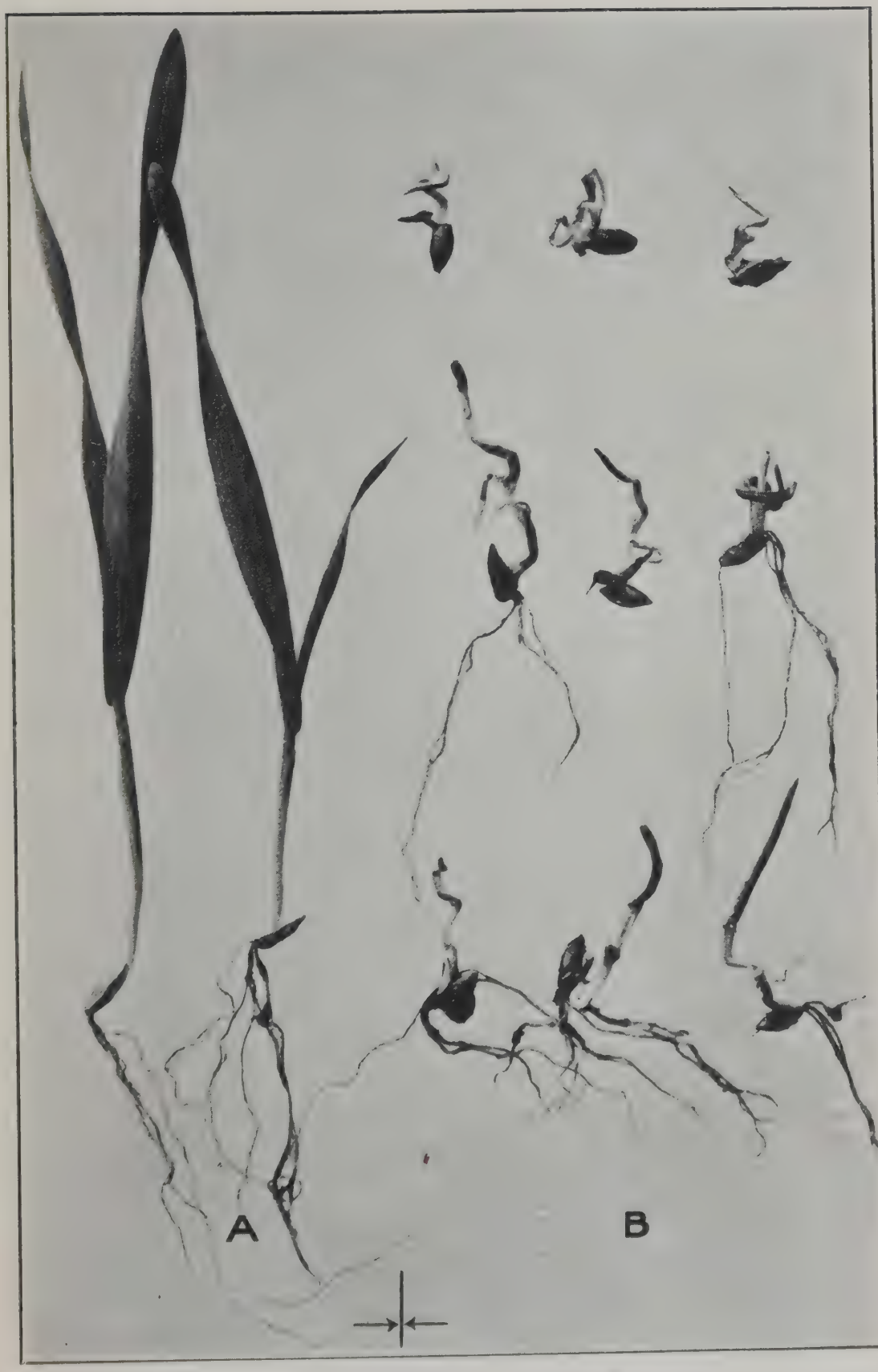
A set of experiments was planned for the purpose of making a study of the effects of the loose-smut fungus on the germination of the seed and the emergence of the seedlings of barley. Seed of two varieties, Texas Winter and Han River, from the 1921 crop grown on Arlington Experiment Farm was dehulled. A part of the dehulled seed of each variety was inoculated and sown and a part sown without inoculation. It was sown about 2 inches deep in soil on greenhouse benches at Arlington Experiment Farm, on January 29, 1924. Close observation every few days revealed a noticeable difference in the number of seedlings emerging from the inoculated and uninoculated seeds. Stands were much better from the latter. Texas Winter germinated very poorly in either case. After several days seeds which failed to produce plants were dug up. Many of the seeds of Texas Winter had decayed, due to the presence of other organisms. In Han River, however, where the uninoculated seed germinated well and produced a good stand of normal plants (Pl. 2, A), the inoculated seeds were found to be germinated but the seedlings were developing very abnormally (Pl. 2, B). The coleoptiles often were much shortened, thick, and tough. For this reason, apparently, they failed to open normally at the tip and the plumule was held within, causing it to curl into various shapes which prevented emergence. Many seedlings which did emerge came through the soil at an angle instead

EXPLANATORY LEGEND FOR PLATE 2

Injury to seedlings of Han River barley by *Ustilago nuda*. Seed dehulled and sown in soil on greenhouse bench. Jan. 29, 1924.

A.—Healthy seedlings from uninoculated seed; 93.44 per cent of the seed produced plants of this type, which were smut-free at maturity.

B.—Seedlings from inoculated seed infected by *U. nuda*. Only 11.29 per cent of the seed produced seedlings sufficiently normal to emerge. The remainder of the seeds germinated but the seedlings were too abnormal to emerge. The infected coleoptiles failed to open up normally and the seedlings became twisted in various ways.



Infection of Barley by Ustilago nuda

Plate 2

(For explanatory legend see p. 268)

of erect, as previously mentioned, and were yellowish in appearance. Some of these later developed a somewhat stiff appearance with a rather bluish-green color. A high percentage of the plants which matured from the inoculated seed developed loose-smut. (Table III.)

On February 13, 1924, another set of dehulled seed of Han River from the 1922 crop was sown about ¾ of an inch deep in a flat in the greenhouse. The seedlings emerged but slightly better from the uninoculated seed. Most of the plants in this flat were used for infection studies and were not grown to maturity, so there are no smut records available. (Table III.)

TABLE III.—*Effects of Ustilago nuda on the emergence of barley seedlings from dehulled seed, and the occurrence of loose-smut in the surviving plants*

Variety	Treatment	Number of seed sown	Number of plants emerged	Per cent of emergence	Number of plants matured	Number of plants smutted	Per cent of smutted plants
Texas Winter	Inoculated	56	3	5.36	3	3	100
Do	Uninoculated	57	20	35.09	11	0	0
Han River	Inoculated	62	7	11.29	5	4	80
Do	Uninoculated	61	57	93.44	57	0	0
Do	Inoculated	50	44	88.00			
Do	Uninoculated	50	46	92.00			

The results given in Table III are very striking. Even though the stands of plants were much poorer from inoculated seed, those plants which survived showed a high percentage of loose-smut. Emergence of seedlings in the flat where the seed was sown ¾ inch deep was much better than was the emergence from seed sown 2 inches deep on the benches. These results led to the belief that the degree of injury to the seedlings by infection would be indicated to a certain extent by the ability of the infected seedlings to emerge when the seed was sown at different depths after inoculation.

A set of experiments was arranged in which the seed was spaced carefully in the rows and covered at uniform depths of ¾ and 1½ inches, respectively, to determine the effects of these depths of sowing on the emergence of the seedlings. It seemed probable that the deeper sowing would produce poorer stands, judging from previous results, both as to stands and the effects of the fungus on the young seedlings. Seed of three varieties of barley—Tennessee Winter, Han River, and Nakano Wase—was dehulled and 25 inoculated and an equal number of uninoculated seed of each was sown in flats in the greenhouse on February 25, 1924, at each of the two depths—¾ of an inch and 1½ inches. A compact soil with little organic matter was used. Four days previous to this, 50 seeds each of inoculated and uninoculated Han River barley were sown in a flat of loose organic soil in the same greenhouse.

Through subsequent observation, it was found that there was a striking difference in the percentage of emergence of seedlings from inoculated seed sown at the two different depths, except in the loose soil, while there was very little difference in the emergence from uninoculated seed sown at different depths (Pl. 3, A to H, Pl. 4, A to D). The surviving plants from the inoculated seed of Tennessee Winter and Han River showed a high percentage of loose-smut (Pl. 5, A and C; Pl. 6, A and C; Pl. 7, A); Nakano Wase from inoculated seed remained smut-free, as did all the plants from uninoculated seed (Pl 5, B and D; Pl. 6, B and D, and Pl. 7, B). (Table IV.)

EXPLANATORY LEGEND FOR PLATE 3

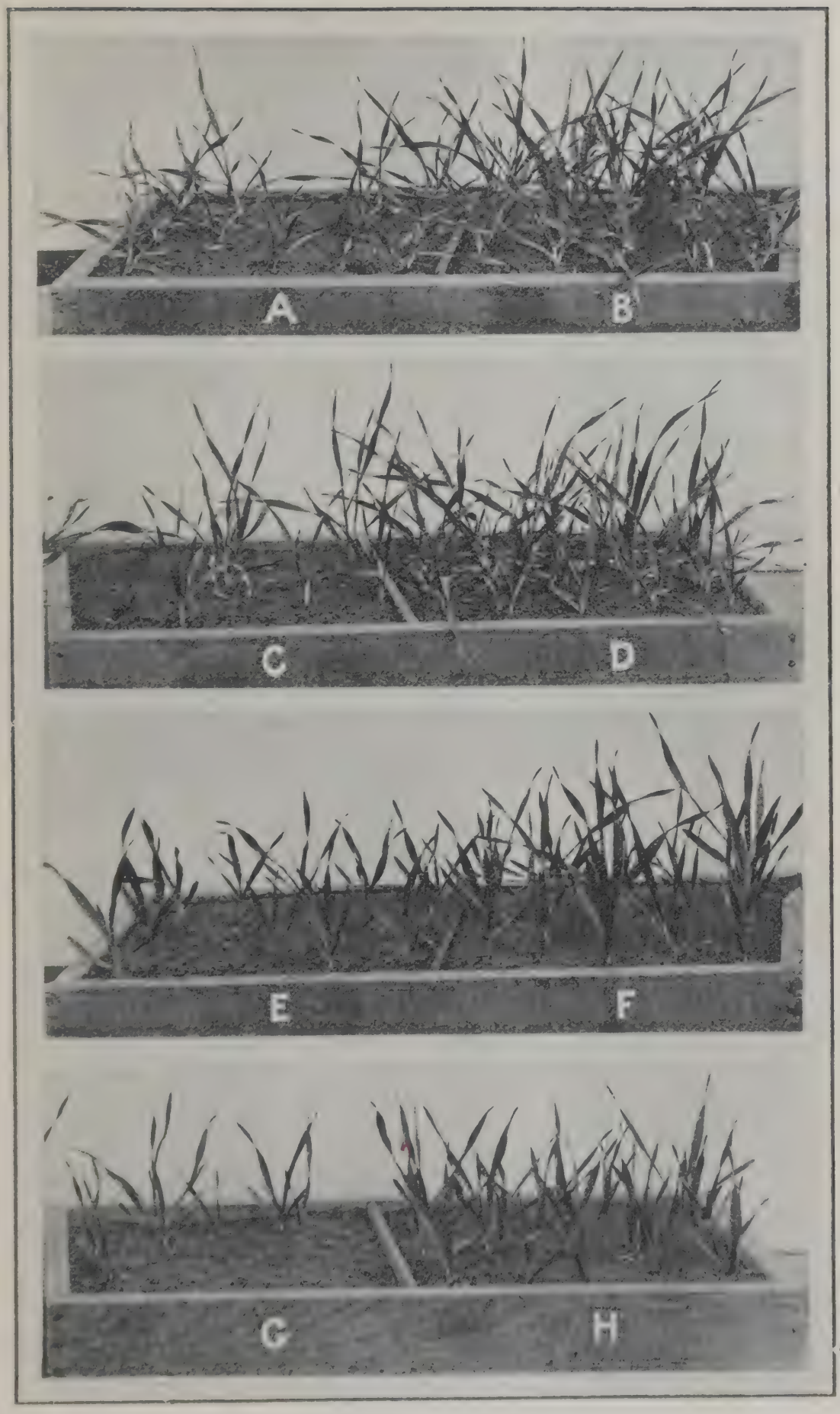
Infection of barley seedlings from dehulled seed by *Ustilago nuda*, and emergence of seedlings from seed sown at different depths in flats in the greenhouse on Feb. 25, 1924.

Tennessee Winter:

A.—Inoculated, sown ¾ of an inch deep; 56 per cent emergence.
B.—Uninoculated, sown ¾ of an inch deep; 88 per cent emergence.
C.—Inoculated, sown 1½ inches deep; 32 per cent emergence.
D.—Uninoculated, sown 1½ inches deep; 84 per cent emergence.

Nakano Wase:

E.—Inoculated, sown ¾ of an inch deep; 60 per cent emergence.
F.—Uninoculated, sown ¾ of an inch deep; 92 per cent emergence.
G.—Inoculated, sown 1½ inches deep; 28 per cent emergence.
H.—Uninoculated, sown 1½ inches deep; 92 per cent emergence.



Infection of Barley by Ustilago nuda

Plate 3

(For explanatory legend see p 270)

99183—25†——2

TABLE IV.—*Effects of Ustilago nuda on the emergence of barley seedlings from dehulled-inoculated seed sown at two different depths, and the occurrence of loose-smut in the surviving plants*

Variety	Treatment	Depth sown in inches	Number seedlings from 25 or 50 seeds	Percentage of emergence	Number of plants smutted	Percentage of smut
			From 25 seeds			
Tennessee Winter	Inoculated	¾	14	56	11	78.57
Do	Uninoculated	¾	22	88	0	0
Do	Inoculated	1½	8	32	8	100.00
Do	Uninoculated	1½	21	84	0	0
Nakano Wase	Inoculated	¾	15	60	0	0
Do	Uninoculated	¾	23	92	0	0
Do	Inoculated	1½	7	28	0	0
Do	Uninoculated	1½	23	92	0	0
Han River	Inoculated	¾	17	68	14	82.35
Do	Uninoculated	¾	24	96	0	0
Do	Inoculated	1½	[a] 4	16	2	100.00
Do	Uninoculated	1½	24	96	0	0
			From 50 seeds			
Do	Inoculated	¾	37	74	34	91.89
Do	Uninoculated	¾	47	94	0	0

[a] Two died.

The data in Table IV show that there was a marked reduction in emergence of seedlings from dehulled-inoculated seed. This was true for all three varieties. The stands were much poorer from inoculated seed sown 1½ inches deep than from similarly treated seed sown only ¾ of an inch deep. The seedlings of Nakano Wase, a variety which has not been known to become smutted, were injured as severely (Pl. 3, E and G) as those of the susceptible varieties, Tennessee Winter and Han River (Pl. 3, A and C and Pl. 4, A and C). The seedlings from the inoculated seed showed the same abnormalities as previously mentioned. They were twisted, and yellowish in color, and some emerged at an acute angle to the soil surface. After emergence the leaves appeared somewhat stiff and later became bluish green in color, and waxlike streaks were noticeable, especially on the dorsal surface of the first two leaves on some of the abnormal plants.

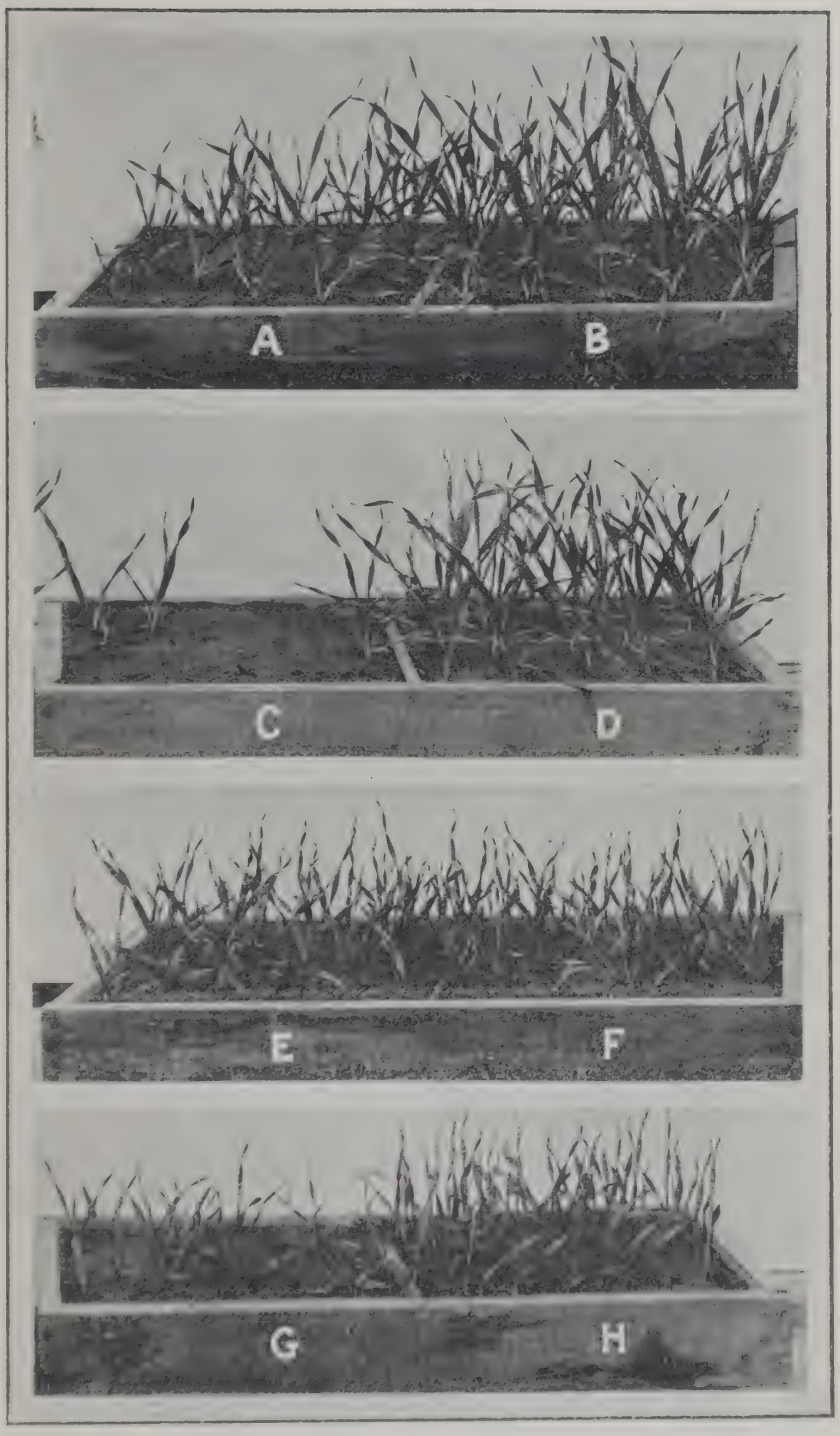
Cause of Seedling Injury

After it had been definitely shown that *Ustilago nuda* caused considerable injury to barley seedlings grown from dehulled-inoculated seed, the question arose as to whether this injury was due to infection and invasion of the tissues of the seedlings by the fungus or to the presence of large quantities of spores on the naked germinating seed, which might have a toxic effect. The experimentation along this line was very limited. It may be well, however, to present the meager evidence obtained. The fact that plants grown from inoculated seed produced high percentages of loose-smut was sufficient evidence that infection of the seedlings took place. Actual penetration of the seedlings by the mycelium of the fungus also had been found in cytologic studies to be discussed later.

A quantity of viable spores of *Ustilago nuda* were placed in a test tube with sufficient water to immerse them. The tube was corked and a standardized thermometer inserted through the cork with the bulb extending into the solution. The tube was then immersed for 15 minutes in water that ranged in temperature from 59 to 61° C. This was done to devitalize the spores and at the same time, if possible, to prevent heating sufficiently high to destroy any

EXPLANATORY LEGEND FOR PLATE 4

Effects of viable and devitalized spores of *Ustilago nuda* on seedlings of Han River barley sown in flats in the greenhouse Feb. 25 and Mar. 8, 1924. Seed all dehulled except those producing plants shown in H.
A.—Inoculated, sown ¾ of an inch deep; 68 per cent emergence.
B.—Uninoculated, sown ¾ of an inch deep; 96 per cent emergence.
C.—Inoculated, sown 1½ inches deep; 16 per cent emergence.
D.—Uninoculated, sown 1½ inches deep; 96 per cent emergence.
E.—Uninoculated, sown 1½ inches deep; 86 per cent emergence.
F.—Inoculated with devitalized spores; sown 1½ inches deep; 94 per cent emergence.
G.—Inoculated with viable spores; sown 1½ inches deep; 48 per cent emergence.
H.—Hulled; inoculated with devitalized spores; sown 1½ inches deep; 98 per cent emergence.



Infection of Barley by Ustilago nuda

Plate 4

(For explanatory legend see p. 272)

toxine that the spores might contain. The spores were then filtered from the solution and left on the filter to dry. A germination test proved these spores to be dead. The untreated spores germinated more than 80 per cent.

Five dehulled seeds of Han River barley inoculated with the devitalized spores and sown on moist sterile filter paper in a Petri dish showed no signs of abnormality while some of the seedlings from seed similarly treated but inoculated with viable spores showed some of the abnormal symptoms observed in seedlings from seed inoculated with viable spores and sown in the soil. The abnormal seedlings were found through microscopic examination to be heavily infected by *Ustilago nuda*.

On March 8, 1924, duplicate lots of hulled and dehulled seed of Han River and Nakano Wase barleys from the crop of 1921 were inoculated according to the regular method, one lot of each with devitalized spores and the second lot with viable spores of *Ustilago nuda*. A third lot was left uninoculated. All lots of the seed were sown 1½ inches deep in flats in the greenhouse. This seed was known to have a slight amount of natural infestation (Table V), but not sufficient to interfere seriously with the results as is shown by the controls in Table V. Seed of Red Wave wheat, one lot inoculated with devitalized and another with viable spores of *U. nuda*, and of an uninoculated control, was sown in similar flats to determine the effect of these spores on wheat seedlings. If the injury of the barley seedlings were due to a toxin produced on the surface of the seed by either the germinated or ungerminated spores, wheat seedlings might also be injured by inoculating the seed with these spores of *U. nuda*.

The emergence of the barley seedlings from dehulled seeds inoculated with viable spores was poor (Pl. 4, G, and Pl. 8, E), as had been previously noted with that type of inoculation. The spores killed by heat had no visible effect on the seedlings (Pl. 4, F and H, and Pl. 8, D and F). The stands were as good as those of the control (Pl. 4, E, and Pl. 8, B, C, and H). A high percentage of loose-smut developed in Han River plants from the dehulled seed which had been inoculated with viable spores (Pl. 7, C). More smut developed in plants from hulled seed of Han River inoculated with viable spores than occurred in corresponding plants from uninoculated seed. Plants from seed inoculated with devitalized spores were smut-free (Pl. 7, D). None of the wheat seedlings showed any signs of injury regardless of how the seed was treated. The emergence was equally good in each case and the plants were normal at maturity. (Table V.)

TABLE V.—*Effects of viable and devitalized spores of Ustilago nuda on barley and wheat seedlings from seed sown 1½ inches deep in the greenhouse*

Variety	Treatment previous to inoculation	Type of spores used	Number of plants from 50 seeds	Per cent of emergence	Number of smutted plants	Per cent of smut
Han River	Dehulled	None	43	86	1	2.33
Do	do	Devitalized	47	94	2	4.26
Do	do	Viable	24	48	24	100.00
Do	Hulled	None	49	98	3	6.12
Do	do	Devitalized	44	88	0	0
Do	do	Viable	48	96	5	10.42
Nakano Wase	Dehulled	None	43	86	0	0
Do	do	Devitalized	41	82	0	0
Do	do	Viable	16	32	0	0
Do	Hulled	None	46	92	0	0
Do	do	Devitalized	45	90	0	0
Do	do	Viable	46	92	0	0
Red Wave (wheat)	No treatment	None	48	96	0	0
Do	do	Devitalized	49	98	0	0
Do	do	Viable	49	98	0	0

EXPLANATORY LEGEND FOR PLATE 5

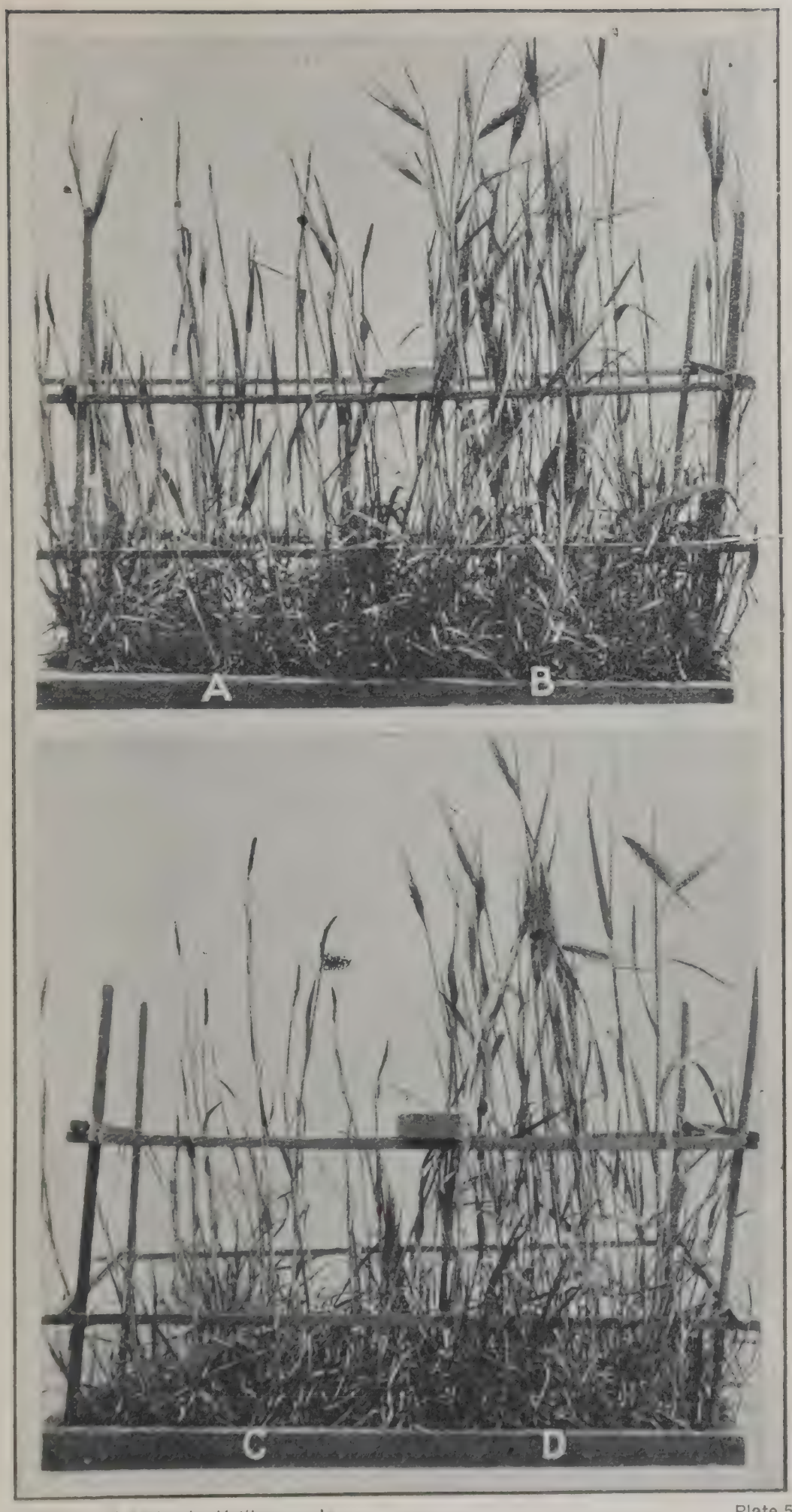
Infection of Tennessee Winter barley from dehulled seed by *Ustilago nuda*. Same plants shown in Plate 3, A to D.

A.—Seed inoculated. Plants 78.57 per cent smutted.

B.—Seed uninoculated. No smut.

C.—Seed inoculated. Plants 100 per cent smutted.

D.—Seed uninoculated. No smut.



Infection of Barley by Ustilago nuda

Plate 5

(For explanatory legend see p. 274)

The data in Table V show that injury to the seedlings was not caused by spores of *Ustilago nuda* devitalized in hot water. They show that neither viable nor devitalized spores had any noticeably injurious effect on seedlings of Red Wave wheat. The viable spores had the same injurious effect on barley seedlings which has been shown in previous experiments (Pl. 4, G and Pl. 8, E). The surviving plants of Nakano Wase from seed dehulled and inoculated with viable spores were smut-free, while those of Han River were 100 per cent smutted (Pl. 7, C). This agrees with the results of previous experiments.

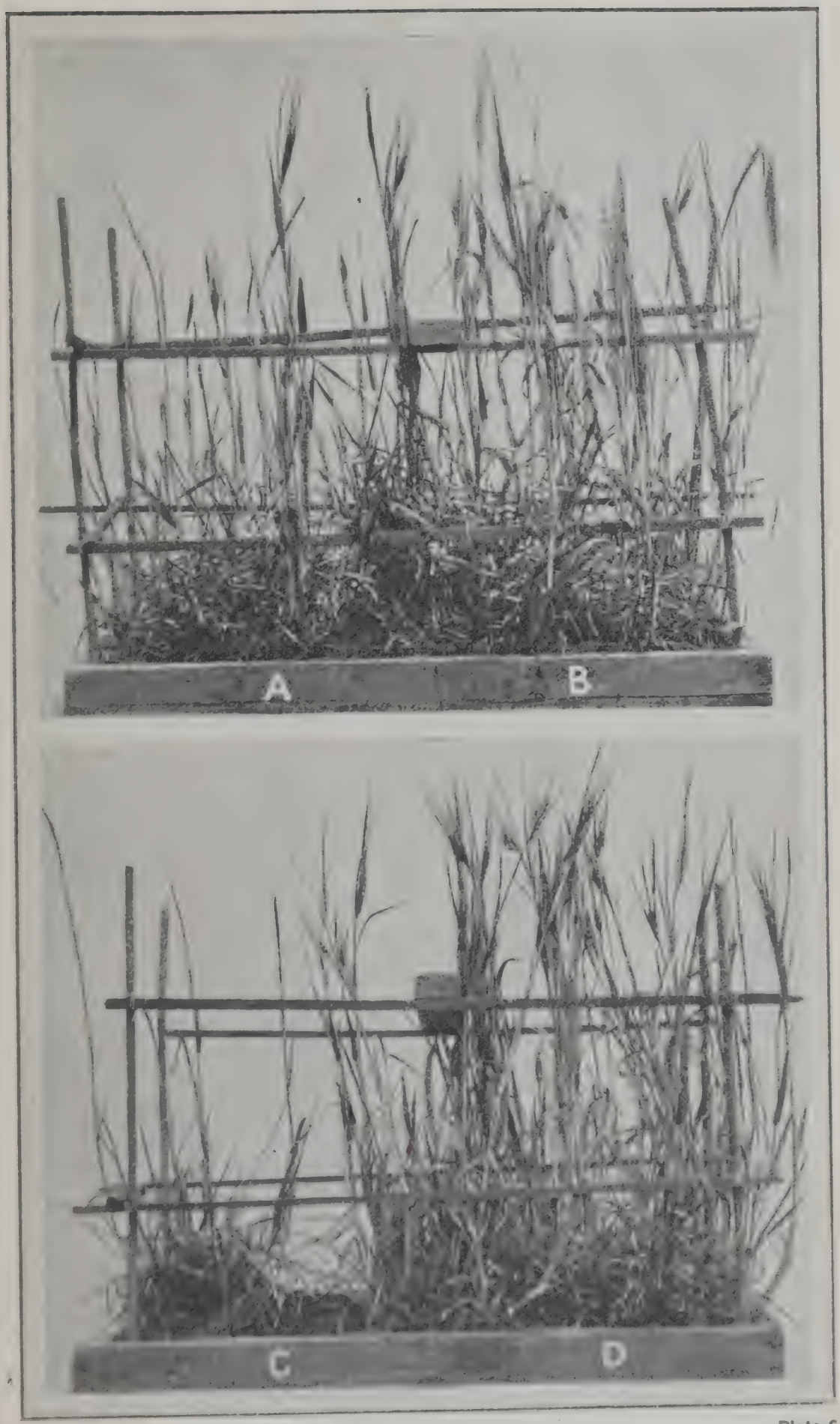
CYTOLOGIC EVIDENCE OF SEEDLING INFECTION

After noting the injurious effects of *Ustilago nuda* on the seedlings of barley, it was decided to make in addition to the studies of possible toxicity, a microscopic study of seedling infection to obtain actual proof of infection and to learn the possible relationship of the amount of infection to seedling injury. Seedlings that failed to emerge from inoculated seed in some of the previous experiments were dug up for this study. Dehulled seed was inoculated and sown in greenhouse flats to obtain further material for this examination. The abnormal seedlings were carefully removed from the soil by taking a clump of dirt with the roots. They were then carefully washed to remove as much of the dirt as possible without any rubbing. The coleoptile was removed from the young seedling with a scalpel and placed on a microscopic slide with the inner surface downward. A few drops of water were then placed on the specimen and pains taken to spread the coleoptile so that it did not fold when the cover glass was gently pressed over it. Glycerin was then added, a drop occasionally, and the slides remained in good condition for study for several days. These slides were studied carefully under the microscope. The coleoptile is sufficiently translucent so that the cellular structure is easily visible with the proper manipulation of the microscope. After considerable search a germinated spore was found adhering to the epidermis of the coleoptile, and the mycelium had penetrated the epidermal cell wall and passed through two or three cells beneath (Pl. 9, *j*). The spore wall was sufficiently intact so that it could be identified as a spore of *U. nuda* by the echinulations on its surface. Many cases of penetration were detected by this method on the seedlings of different varieties of barley including Nakano Wase which has never produced smutted heads in the writers' experiments (Pl. 9, *c*, *d*, *f*, *i*, *j*, and *l*). A more direct method was then employed for studying penetration, one in which the possibility of contamination was reduced to a minimum and in which no washing of the seedlings was necessary before the examination was made. A method similar to the one employed by Tisdale (*18*) in his studies of the penetration of the root hairs of flax seedlings by *Fusarium lini* was used. As the smuts infect the seedlings of small grains so early in their development, this method was considered satisfactory. The seedling is not far enough advanced to be drawing much, if any, of its food from the soil at the time when infection takes place. The seedlings apparently are in normal condition in the tube cultures at this age.

Strips of paper towel were rolled and inserted into test tubes, the rolls being one-third the length of the tubes, and enough water added to wet the paper thoroughly and to fill the tube up to about one-half the depth of the paper roll. The tubes were sterilized in an autoclave. Dehulled and hulled barley seeds were treated by the modified hot-water method to kill any smut spores or other fungi on or in them. A part of the seed was then inoculated with spores of *Ustilago nuda* which had been collected from Tennessee Winter barley the previous year and kept in a well-corked bottle after drying. This inoculated seed and a similar lot of uninoculated seed were sown in these sterile tubes. The tubes were placed in a dark closet at room temperature (about 21° C.) and left for two days, at the end of which time the inoculated seeds which were germinating had a frosty appearance. When examined under the microscope this whitish substance proved to be the mycelium of germinated spores of *U. nuda*. Coleoptiles of these seedlings examined according to the method previously

EXPLANATORY LEGEND FOR PLATE 6

Infection of Han River barley, from dehulled seed, by *Ustilago nuda*. Same plants shown in Plate 4, A to D.
A.—Seed inoculated. Plants 82.34 per cent smutted.
B.—Seed uninoculated. No smut.
C.—Seed inoculated. Plants 100 per cent smutted.
D.—Seed uninoculated. No smut.



Infection of Barley by Ustilago nuda

Plate 6

(For explanatory legend see p. 276)

described showed no signs of infection. Further examinations were made on the fourth day and some of the seedlings were found to be heavily infected by these hyphae (Pl. 9, *a*, *b*, *e*, *g*, *k*, and *m*). There was no mistake as to the identity of the fungus. Similar cultures were made in Petri dishes on sterile paper with the same result. The seedlings were easy to remove for study and, as with the tube cultures, no washing was necessary before the examination was made. In this way the spores were not disturbed and could be found attached to the hyphae. Infections of seedlings of the following varieties were found by this method: Tennessee Winter, Han River, Nakano Wase, and Smyrna (C. I. 195), a two-rowed barley. In one case the first two leaves of a seedling of Han River were found to be heavily infected (Pl. 9, *h* and *n*). Nakano Wase, the variety which has not shown any smut at maturity, apparently was infected as easily as the susceptible varieties (Pl. 9, *k* and *m*). One hull-less barley, C. I. 2222 was sown in the dish cultures and examined to a limited extent but no infections were found. Reports indicate that the naked barleys smut heavily in nature.

In many cases the mycelium was found entering the epidermis at or near the wall between two cells (Pl. 9, *b*, *e*, *g*, *j*). This was not always true, however, as the hyphae seemed to be able to penetrate the cell wall at any point (Pl. 9, *a*, *d*, *f*, *i*). The fact that the fungus is able to infect the coleoptile and, in cases, the first leaves, is not proof that it will reach the growing point of the seedling and finally result in a smutted plant. However, the injury caused to the seedling (Pl. 3, 4, 7, and 8) is a very good indication that its activities are not confined to the coleoptile and the superficial tissues of the plant. Furthermore, the high percentages of loose-smut occurring in the plants of susceptible varieties grown from inoculated seed is definite proof that the fungus reaches the growing point of the seedling and keeps pace with its development. With Nakano Wase the fungus evidently penetrates beyond the superficial layers, as evidenced by severe seedling injury (Pl. 3 and 8) but is unable for some reason to keep pace with the development of the plant. Consequently, the surviving plants are smut-free at maturity. The seedling injury no doubt is due to heavy infection and invasion of the tissues of the plant by the fungus mycelium. If a toxin actually is responsible for the injury it evidently is produced within the tissues of the host as invasion takes place.

DISCUSSION

Floral infection has been recognized as the only means of infection of barley by the loose-smut fungus, *Ustilago nuda*, for many years. No doubt, if Jensen (*12*) had continued his infection studies with dehulled seed and included the loose-smut fungus after discovering it he would have found that it could infect the seedlings of barley. Maddox (*16*) through whose work floral infection was first established, apparently overlooked the possibility of the spores remaining viable between the glumes and the kernel when the flower is inoculated. He failed to consider the fact that the hulls furnish a very effective obstruction to infection. This is true for both barley smuts. Since the time of Maddox other investigators have followed practically the same methods of inoculation used by him, with the exception of Brefeld (*3*) in a part of his investigations. Brefeld not only inoculated the flowers but also inoculated young seedlings with germinated spores. With the latter method he secured 1 per cent of infection in one experiment. This might have been due to natural infection or perhaps to contamination, but there is a chance that it was due to the inoculation. Perhaps Brefeld's method of inoculation or else the treatment of the spores, or seedlings, or both, previous to inoculation had upset their normal functions. The spores were first germinated in water and then finely divided in a nutrient solution before spraying on the seedlings. There no doubt are many reasons why they might not have functioned normally.

Other investigators have inoculated the flowers at various stages of development and have found that more infection takes place if the flower is inoculated in one stage than if it is inoculated in another. It seems entirely possible that the weather condi-

EXPLANATORY LEGEND FOR PLATE 7

Effects of viable and devitalized spores of *Ustilago nuda* on Han River barley. C and D were shown in Plate 4.

A.—Seed dehulled and inoculated. Plants 91.89 per cent smutted.
B.—Seed dehulled and uninoculated. No smut.
C.—Seed dehulled and inoculated. Plants 100 per cent smutted.
D.—Seed hulled and inoculated with devitalized spores. No smut.

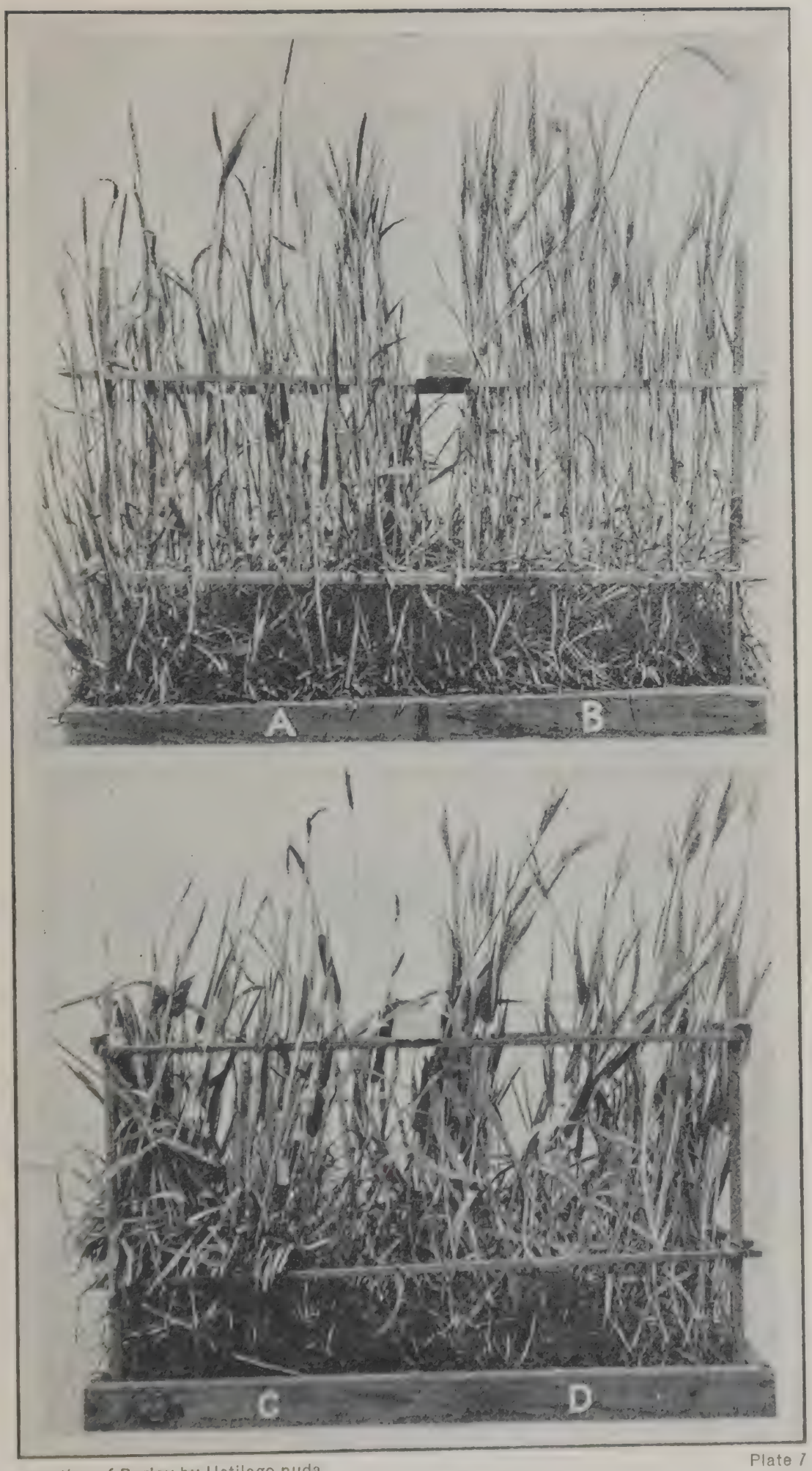


Plate 7

Infection of Barley by Ustilago nuda

(For explanatory legend see p. 278)

tions at the time of inoculation might determine whether the spores would germinate immediately, or remain ungerminated behind the glumes. The age and condition of the spores might also be a determining factor. In our studies low percentages of infection were obtained by inoculating the flowers, and the stage the flowers were in when inoculated made little difference in the amount of infection obtained.

Hecke (*9*) illustrates a mycelium in the tissues of the scutellum of barley kernels. It does not seem entirely impossible that Hecke was mistaken in the identity of the fungus. On the other hand, it is possible that the fungus infects at flowering time to a certain extent. Certain local climatic conditions might be more conducive to floral infection. Should floral infection take place there still remains the possibility that the internal mycelium would not become active when the seed is sown. In this respect the control by superficial disinfection is significant.

There may be specialized strains or races of the fungus *Ustilago nuda* such as have been found in other fungi, including some of the cereal smuts (*6* and *17*). This seems to be a very logical conclusion, in view of the fact that there is a recognized tendency on the part of living organisms to adjust themselves to their environment. In the higher plants not only varieties and strains but distinct individual differences within the variety or strain are found. It is not beyond possibility that there are strains of the loose-smut fungus which infect through the flower.

Brioli (*4*), working with four varieties of barley, says that he found mycelium of *Ustilago nuda* in the scutellum of the seed of only one variety. He was inclined to the opinion that the other three varieties were resistant. The lack of infection might have been more nearly what happens in nature. However, the varietal differences of the host plant may be a determining factor. Some varieties may be more susceptible to this type of infection.

The investigations recorded herein have shown that seedling infection by *Ustilago nuda* takes place abundantly in certain varieties of barley when the dehulled seed is inoculated. Infection is so heavy in many cases as severely to injure the young seedling. The rate of emergence of the seedlings from seed sown at different depths indicates the amount of injury done to the seedlings. An injured seedling is more capable of emerging through thin than it is through thicker layers of soil within the depths at which sowing is customary. Loose soils are more favorable to emergence than are close, compact soils. There may be a toxic effect of the fungus on the host but the data indicate that if a toxin exists it is produced internally after the plant is infected. There are no indications of injury due to the presence of spores on the seed.

Seedlings of both susceptible and resistant barleys are injured by the fungus. Infection studies have shown that both types become infected. The germ tube is capable of penetrating directly the cell walls of the coleoptile and at least the first two leaves of the young seedlings. The surviving plants from inoculated seed produced a high percentage of heads smutted with loose-smut. Nakano Wase is an exception. It failed to become smutted in all experiments and apparently is highly resistant to invasion by the fungus after it enters the seedling. These investigations have shown conclusively that seedling infection by *Ustilago nuda* will take place in a number of varieties of barley and have shaken the long-accepted theory that floral infection is the only type of barley infection by the loose-smut fungus, *U. nuda*.

SUMMARY

Floral infection of barley by *Ustilago nuda* has been accepted as proved for many years.

Surface disinfectants have been found to control loose-smut in certain varieties of barleys.

Dehulled seed of a number of varieties of barleys inoculated with spores

EXPLANATORY LEGEND FOR PLATE 8

Effects of viable and devitalized spores of *Ustilago nuda* on Han River and Nakano Wase barley seedlings grown from hulled and dehulled seed sown 1½ inches deep in flats in greenhouse, on Mar. 8, 1924.

Han River:

A.—Hulled, inoculated with viable spores; 96 per cent emergence.

B.—Hulled, uninoculated; 98 per cent emergence.

Nakano Wase:

C.—Dehulled, uninoculated; 86 per cent emergence.

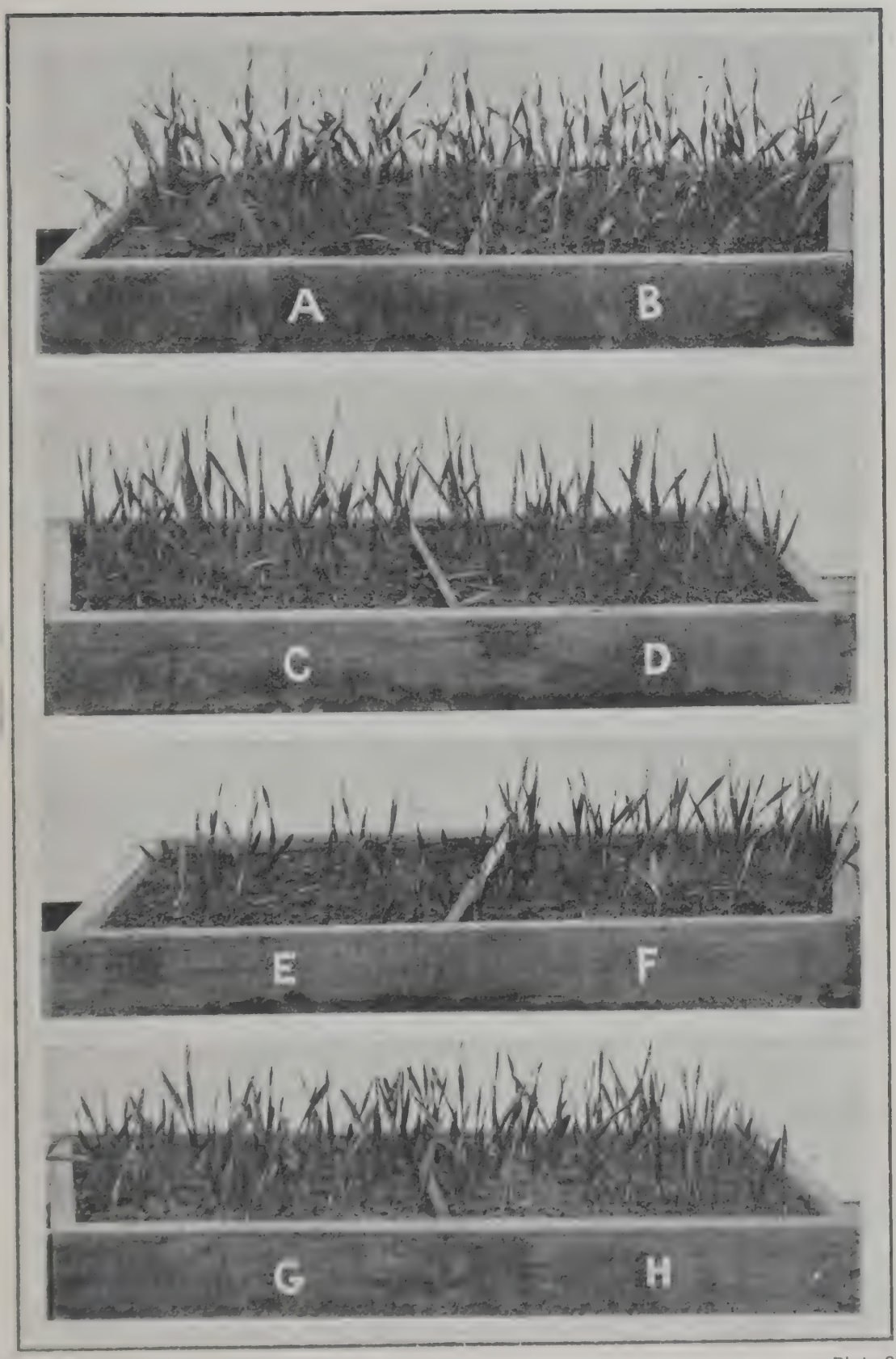
D.—Dehulled, inoculated with devitalized spores; 82 per cent emergence.

E.—Dehulled, inoculated with viable spores; 32 per cent emergence.

F.—Hulled, inoculated with devitalized spores; 90 per cent emergence.

G.—Hulled, inoculated with viable spores; 92 per cent emergence.

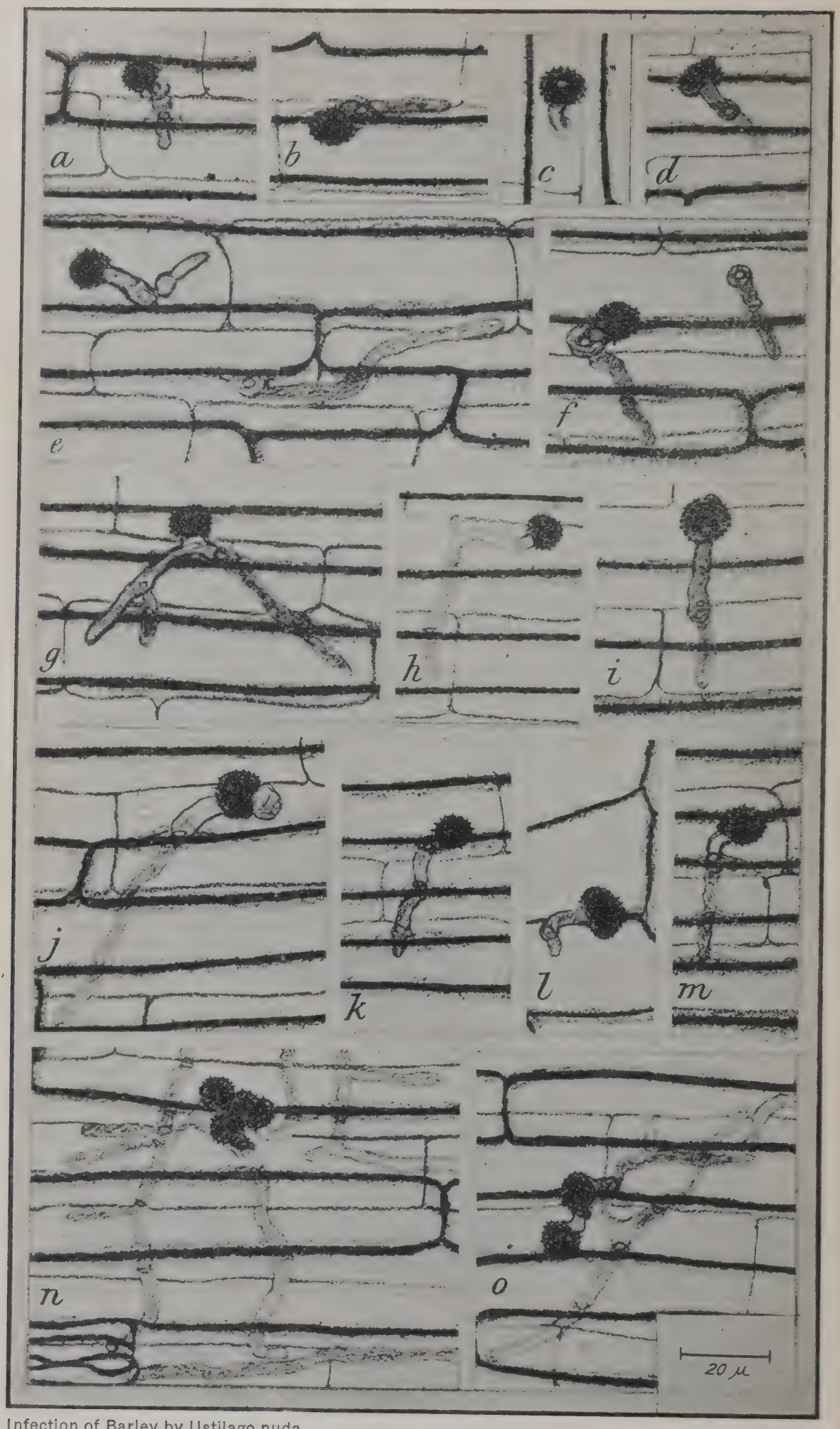
H.—Hulled, uninoculated; 92 per cent emergence.



Infection of Barley by Ustilago nuda

Plate 8

(For explanatory legend see p. 280)



Infection of Barley by Ustilago nuda

Plate 9

(For explanatory legend see p 283)

of *Ustilago nuda* produced plants with a high percentage of loose-smut.

Nakano Wase barley remained smut-free even though the seed was dehulled and smutted.

Seedlings from dehulled-inoculated seed of all varieties of barley studied were severely injured, and many of them failed to emerge.

Seedlings from dehulled-inoculated seed sown three-fourths of an inch deep emerged better than those from similar seed sown 1½ inches deep.

Seedlings from hulled-inoculated seed were not noticeably injured.

Devitalized spores of *Ustilago nuda* were not harmful to barley seedlings grown from the inocluated seed.

Neither viable nor devitalized spores of *Ustilago nuda* were harmful to seedlings of Red Wave wheat grown from inoculated seed.

A miscroscopic study revealed infection of the coleoptile and first leaves of the plumule of barley seedlings by *Ustilago nuda*. Both susceptible and resistant varieties were infected.

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EXPLANATORY LEGEND FOR PLATE 9

Infection of barley seedlings by *Ustilago nuda*.
Coleoptile infection in sterile tube and dish culture: *a*, Smyrna (C. I. 195), a two-rowed barley; *b*, *c*, and *g*. Nakano Wase; *k* and *m*, Tennessee Winter; *o*, Han River.
Coleoptile infection in soil: *c*, *d*, *f*, and *i*, Han River; *j*, Tennessee Winter. Infection at base of coleoptile, in soil; *l*, Han River.
Infection of first leaf in sterile dish culture: *h* and *n*, Han River.

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THE EFFECT OF FEEDING THYROID ON THE PLUMAGE OF THE FOWL [1]

By L. J. Cole, *Chairman, Department of Genetics, University of Wisconsin,* and D. H. Reid, *Head Professor of Poultry Husbandry, Agricultural and Mechanical College of Texas*

The assumption of male plumage by the female fowl has frequently been observed and commented upon. When it occurs in nature it is usually found associated with a disturbance of the ovary (*1*),[2] and it may be produced experimentally as a sequel of ovariotomy (*3*). The writers are not aware that the spontaneous assumption of female plumage by the male fowl has been reported (leaving out of consideration those breeds, such as the Seabright Bantam, in which it is a normal inherited characteristic), though it is a process which occurs annually in many dimorphically colored birds, such as the scarlet tanager, bobolink, and indigo bunting. Goodale (*4, 5*) has succeeded, however, in inducing henfeathering in males by a combination of castration and implantation of ovarian tissue. More recently, Torrey and Horning (*6*) have reported that when dried thyroid was fed to normal growing male chicks they developed female plumage instead of that natural to their sex. No change of plumage was effected, however, by feeding thyroid to normal females nor to castrated males or females. These results are of interest and seem important, even though a trial reported by Crew and Huxley (*2*) has failed to confirm them. It would seem that for the present the accumulation of additional evidence is more to be desired than extended discussion of the possible action of glandular secretions on the development and expression of secondary sexual characters.

A preliminary trial of the effects of feeding thyroid to adult males was made by the writers at the Wisconsin Agricultural Experiment Station in the late winter and early spring of 1923. Adult males were used for the test, partly because no chicks were available at the time and partly to see whether or not any effect would be visible in the replaced feathers of the grown bird.

Ten Brown Leghorn cockerels nearly a year of age and one old male were used in the experiment. All had normal plumage of the breed with respect both to color and form of feathers. They were placed in individual coops (about the size of exhibition coops) in the poultry building, but were divided into four lots with respect to treatment, as follows:

In addition to the regular ration—

Lot 1, consisting of four birds, received every other day at first and later, daily, 400 mgm. of desiccated thyroids[3] containing 0.2 per cent iodine, or 0.8 mgm. iodine per dose. Torrey and Horning started with 50 mgm. of thyroids when their chicks were four weeks of age and increased the dose from time to time to 330 mgm. at the end of 15 weeks. They do not state the weights of their birds, but the writers have assumed that these weighed about 4 pounds when 19 weeks of age, and the dose for the birds in the present experiment, which averaged about 4.75 pounds, was calculated at approximately the same proportion in relation to weight. The material was easily administered in capsules.

Lot 2, two birds, each fed 1.05 mgm. KI, in capsules, the iodine content being equivalent to that in the thyroids. These were fed at the same intervals as the thyroids.

Lot 3, two birds, each given at the same periods by pipette 1 cc. of 0.08 per cent solution of iodine in 28.5 per cent alcohol.

Lot 4, three birds, controls, received no dosage.

The experiment was begun on February 9, all birds being given their doses on succeeding alternate days up to March 1; after that they were dosed daily until the experiment was con-

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[2] Reference is made by number (italic) to "Literature cited," p. 287.

[3] The writers desire to express their appreciation to Armour & Co., Chicago, for their kindness in furnishing the thyroids used in this experiment.

cluded on May 2. On February 20, a patch of feathers was pulled from the cape and wing bow of each bird and saved for future reference. (See Pl. 1.) On March 1, a patch from the saddle and one sickle feather were also pulled. All birds were weighed weekly and notes were kept on general health and on growth of new feathers. Such results as were obtained may be stated briefly.

There was considerable variability in the rate at which new feathers replaced those which were pulled, depending in part, apparently, on the state of health of the individual bird, but also, as will be noted later, on the medication. In the better-developed cases the new feathers were of full length by May 2, but in the others comparatively little replacement had occurred. In no case was the sickle replaced.

It will simplify matters to make the summary statement that in all lots except that receiving thyroids the new feathers were in all respects normal both as to shape and coloration. This is doubtless what was to be expected, since experiments on mammals have shown little effect caused by administering iodine, free or in inorganic combination, except in cases of iodine deficiency or of subnormal activity of the thyroid gland. There is no reason to suppose that either of these conditions existed in the present instance.

The new feathers on the thyroid-fed birds were, however, strikingly different from those which were pulled, as is indicated in the accompanying tabulation. There was considerable variation, as will be noted, and it can not be said that the replaced feathers were characteristically female. As relates to color, there was an evident action toward the reduction of red pigment, varying in degree in the different birds, but tending to be arranged in "stippling" when present. This was particularly true in the new cape feathers of bird No. 441, which were decidedly femalelike in appearance. In all cases the red was broken and much more irregular in distribution than in normal male feathers. The resemblance to female feathers was much more striking in respect to shape and structure, the broad, rounded ends contrasting strongly with the pointed tips of the male plumage on these parts. Furthermore, the great reduction or absence of the zone of "free" barbs, that is, of barbs lacking barbules and hooks, so characteristic of the normal male feathers, was very evident, and constitutes another resemblance to female plumage.

Summing up, it may be said that the feeding of desiccated thyroids to male Brown Leghorn fowls in certain amounts profoundly modifies feathers grown while the material is being administered. The feathers produced under these conditions are not typically female in type, but they do show distinct female characteristics. There would seem to be in this case no question of altering the sex of the bird, nor in all probability of the sex hormones. Nor is it likely that the administered thyroid material acts through the mediation of the thyroid gland of the bird. It seems altogether more probable that the thyroid fed acts directly in influencing the metabolism of the developing feather germ. The very evident tendency to an increased melanism may indicate a higher oxidation of the pigment products; the explanation of the change in form must be less direct.

The feeding of iodine, free and in inorganic combination, gave no result comparable to that obtained from dessicated thyroids containing an equivalent amount of iodine.

One other apparent effect of the thyroid feeding deserves mention, namely, that the replacement of feathers on the birds receiving thyroids was noticeably more rapid than on the others. Since some of the birds were suffering from "colds" and were not in the best of health during the trial, however, it would not be safe to make too definite a pronouncement on this point. If the feeding of thyroid should prove to have a stimulating effect on feather development it might prove useful in inducing quick and uniform molting, a factor of considerable practical importance. It is proposed to test this further by later experiments.

EXPLANATORY LEGEND FOR PLATE 1

A, B, and C.—Normal feathers from saddle, cape, and wing bow, respectively, of bird No. 448. Cape and wing bow feathers pulled February 20; saddle, March 1.

D, E, and F.—New feathers grown by bird No. 448, replacing those pulled (D, wing bow; E, cape; F, saddle). The new feathers, which were pulled on May 2, appear normal in shape, structure, and coloration, apparently not having been affected by the free iodine in alcoholic solution administered to this subject.

G, H, and I.—New feathers (May 2) from saddle, cape, and wing bow, respectively, from bird No. 441. These feathers show modification of shape, structure, and coloration, approaching the female type in these respects. The stippling in the cape feather is closely similar to that which occurs in females. These modifications are attributed to the desiccated thyroids fed.

J and K.—New cape and saddle feathers from bird No. 444, following feeding of desiccated thyroids. The melanistic effect was especially pronounced in this bird.

A
B
C
D
E
F
G
H
I
J
K

COMPARISON OF NEW FEATHERS IN THYROID-FED BIRDS WITH THOSE OF NORMAL BIRDS

	Cape	Wing Bow	Saddle
Normal birds	Exposed portion generally red, base black; red may occupy whole distal half of feather or be reduced to a border. Distinctly pointed; with broad zone of free barbs.	Similar to cape but occasionally with black spot at tip. Same general shape, but shorter.	Varying from almost wholly red to red with black middle stripe. Long (some 12 cm.); narrowly attenuated distally; pointed. Wide zone of free barbs.
Bird No. 441	Black, with *definite red stippling* near tip. Broadly rounded, without zone of free barbs.	Black with a considerable quantity of red more or less irregularly intermixed; tendency to stippling. Broadly rounded, without "free zone."	Black with considerable red irregularly distributed. Elongate (11 cm.) but broad, and rounded at tip; very little "free zone."
Bird No. 440	Black; little if any red. Broadly rounded; without "free zone."	Black. Broadly rounded; without "free zone."	Dull black with some irregular red; tendency to stippling. Broad, rounded, without "free zone"; length 8 cm.
Bird No. 444	Mostly black; a few feathers with a small amount of red on margin. Bluntly rounded; without "free zone."	Black with small amount of red, showing tendency to stippling. Bluntly rounded; no "free zone."	Black (no red). Elongate (11 cm.); with somewhat the taper of male saddle feathers but broader and rounded at tip; narrow "free zone" near tip.
Old male	Black or black with little red. Broadly rounded; without "free zone."	None available. (This bird pulled the new feathers he could reach as they grew.)	None available.

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POLYSCELIS MODESTUS GAHAN, A MINOR PARASITE OF THE HESSIAN FLY [1]

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INTRODUCTION AND HISTORY

The hymenopterous parasite which is the subject of this paper was first reared from the Hessian fly (*Phytophaga destructor* Say) in the summer of 1915 by the late W. R. McConnell and the author at the Hagerstown, Md., Laboratory of the Cereal and Forage Insect Investigations of the Bureau of Entomology, United States Department of Agriculture, when a heavy infestation of that insect pest of wheat occurred generally throughout the wheat-growing region of the Eastern States.

At that time specimens of this species were submitted for determination to A. B. Gahan, of the Bureau of Entomology, who tentatively determined them as *Polyscelis* sp. A subsequent examination of the material and literature, however, revealed it to be a new species and accordingly it was described by Mr. Gahan [3] in 1922 under the name *Polyscelis modestus*.[4]

Although knowledge of this species is rather meager, it seems advisable to record the data which have accumulated concerning its life history, especially in view of the fact that the species is uncommon and no recoveries of it have been made since 1918.

DISTRIBUTION

The first specimens of this species were reared from Hessian fly puparia collected by the author in 1915 near Hanover, Pa. Later on, however, specimens were reared from material collected in the following five additional localities in the States of Maryland and Pennsylvania: Hagerstown, Md. (W. R. McConnell, E. M. Craighead); Andersonburg, Carlisle, Gettysburg, and Perkasie, Pa. (P. R. Myers).

HOSTS

Normally *Polyscelis modestus* is a primary, solitary parasite of the Hessian fly. The primary nature of its attack on this host has been demonstrated on several occasions by breeding it experimentally in the laboratory upon nonparasitized Hessian fly puparia. It attacks the host usually in the larval stage but cases have been found where it has developed also upon Hessian fly pupae. It feeds externally on its host within the puparium. Completing its development within the puparium, it emerges therefrom as an adult.

This species has also been shown to be a secondary parasite by the rearing of a single minute male from a puparium which, when opened, was found to contain the cocoons of a platygasterid parasite, probably *Platygaster vernalis* (Myers).

In another instance two eggs of *P. modestus* were deposited within the same puparium. Shortly after the hatching of these eggs one of the resulting larvae attacked the other and by the following day had consumed its contents. Evidence was also found indicating that young larvae probably attack and destroy the eggs of their own species.

These cases of hyperparasitism and hyperpredacity plainly indicate that upon occasion this species can develop upon parasitic larvae, and probably pupae, of its own or some other species as well as on the larvae and pupae of the Hessian fly.

ECONOMIC IMPORTANCE

Although this species was easily reared upon nonparasitized Hessian

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[2] The biological data contained in this article were obtained principally from experiments conducted by the late W. R. McConnell in the laboratory of Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture, at Carlisle, Pa., during the summer of 1917. The writer gladly expresses his indebtedness to Miss Esther H. Hart for the drawings of the adult and the leg, wings, and antennae of adults; and to C. C. Hill for the drawings of the egg, larva, pupa, and parts of larva, and for kindly criticisms.

[3] GAHAN, A. B., DESCRIPTIONS OF MISCELLANEOUS NEW REARED PARASITIC HYMENOPTERA. Proc. U. S. Nat. Mus., 61: 11-12. 1922.

[4] Order Hymenoptera, superfamily Chalcidoidea, family Pteromalidae.

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fly puparia in the laboratory both at Hagerstown, Md., and Carlisle, Pa., yet in the course of the investigations it has been possible to secure only 13 specimens of this species from material collected in the field. In view of the fact that so few specimens have been reared from material collected in the field, it would seem that this species is one of the rare and less important parasites of the Hessian fly. Adults have been reared from Hessian fly puparia, both of the fall and spring generations. The maximum percentage of parasitism obtained from material of the spring generation was 0.85 per cent, whereas in the material of the fall generation it was 1.5 per cent.

EGG

The egg (fig. 1, *a*) is translucent whitish in color. In shape it is rather ovate with the cephalic end larger and more obtuse than the caudal end. It is about two and one-half times as long as its greatest width. The ventral region is slightly concave and the dorsal region convex. The chorion is thin and elastic and is almost entirely densely spinose. There is a small circular area at the caudal end bare of spines. The spines are of uniform length everywhere except close to the periphery of the smooth area. Here they shorten declivitously. The length of the egg varies from 0.34 to 0.38 mm. and its greatest width varies from 0.14 to 0.15 mm. The average measurement of 10 eggs was 0.36 mm. in length by 0.15 mm. in greatest width.

INCUBATION

During the month of July when these experiments were conducted, the period of incubation varied, occupying from one and one-half to two days. In hatching, the young larva pushes its head against the ventral portion of the cephalic end of the egg, stretching the elastic chorion at this point into a blunt protuberance. After pushing against the chorion for some seconds the larva withdraws into its normal position. This process is repeated after short intervals for an hour or more before hatching actually occurs. During the effort of the larva to break through the chorion the posterior part of the shell is contracted irregularly by the forward movement of the larva, and the mandibles of the young larva can be observed opening and closing within.

PRIMARY LARVA

The newly hatched larva is about 0.35 mm. long, or about the length of the egg itself. It is widest at the cephalic end but tapers gradually to a rather acute point at the caudal end. There are rather prominent tubercles on each side of the anterior body segments, each of which is surmounted by a coarse seta. The head bears a pair of antennae and some fine setae. The mouth, which is of a protruding suctorial form, is readily discernible. In its attack upon its host the young parasitic larva may attach itself to any part of the host's body.

MATURE LARVA

The mature larva (fig. 1, *b*) varies from 1.5 mm. to 2 mm. in length and from 0.5 mm. to 0.6 mm. in width. The average length of 10 larvae was 1.7 mm. and the average width was 0.54 mm. It is of a typical chalcidoid form, somewhat spindle shaped, slightly curved, tapering to both extremities but more acutely toward the caudal than the cephalic end. It is white in color, smooth, shining, and bare except for two rows of fine setae along the laterodorsal area of the first 12 segments. These setae are arranged two to a segment, one directly above the other. The body consists of 13 segments. The anal segment is divided into a dorsal and a ventral lobe, the dorsal lobe being margined posteriorly by a row of four setae. Spiracles are present on the mesothoracic, metathoracic, and the first seven abdominal segments. The head (fig. 1, *c*) when viewed from the front is slightly concave giving it a somewhat bilobed appearance. The antennae which are very short and pale brown in color are surmounted on a fleshy tubercle. The labrum is broadly ovate, slightly curved, and sparsely and finely setose. Two setae are discernible on either side of the head, laterad of the mouth. The mandibles (fig. 1, *d*) of the mature larva are about 0.03 mm. long. They are chitinous, light brown in color, simple in form and nearly straight. The superior margin of the mandible is slightly curved while the inferior margin is nearly straight. No other appendages of the mouth parts are discernible.

The duration of the larval stage varies from 4 to 11 days. The average length of the larval period for 30 larvae was slightly more than 6 days. Table I shows the individual variation of the duration of the larval and other stages.

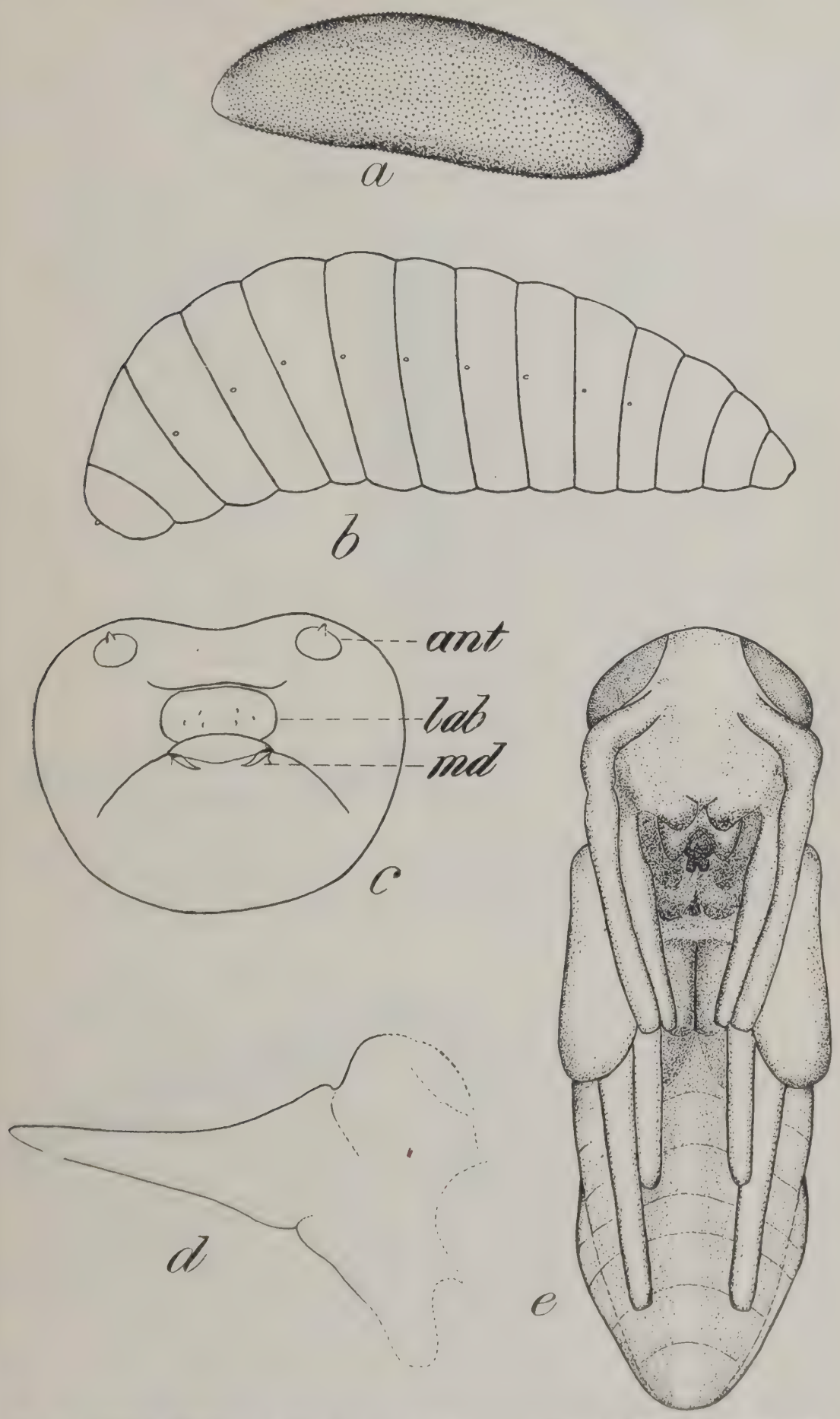

FIG. 1.—*Polyscelis modestus*: *a*, Egg, greatly enlarged (actual length 0.38 mm.); *b*, mature larva, enlarged (actual length 1.6 mm.); *c*, front view of head of mature larva, showing mouth parts much enlarged (*ant*, antenna; *lab*, labrum, *md*, mandible); *d*, mandible of mature larva, greatly enlarged (actual length 0.027 mm.); *e*, pupa, ventral view (actual length 1.8 mm.).

TABLE I.—*Life-history data on Polyscelis modestus* [a]

Host puparium No.	Date of oviposition	Date of meconial discharge	Date of pupation	Length of larval stage	Length of prepupal stage	Date of emergence	Length of pupal stage	Period from egg to adult
				Days	*Hours*		*Days*	*Days*
14	July 18	July 26, 11.45 a. m.	July 27, 2 p. m.	[b] 7	26¼	Aug. 1	5	14
15	18		July 27, 2 p. m.	7		2	6	15
27	19	July 26, 3.30 p. m., at dissection.	July 27, 2 p. m.	6	22½	2	6	14
28	19	July 26, 3.30 p. m., at dissection.	July 27, 2 p. m.	6	22½	2	6	14
29	19	July 26, 3.30 p. m., at dissection.	July 27, 2 p. m.	6	22½	2	6	14
42	22		July 31, 2.30 p. m.	7				
43	22		July 31, 2.30 p. m.	7		7	7	16
47	22	July 27, 2.30 p. m., at dissection.	July 28, 9.30 a. m.	4	19	2	5	11
54	22	July 27, 2.30 p. m., at dissection.	July 28, 9.30 a. m.	4	19	2	5	11
79	24		July 31, 2.30 p. m.	5		6	6	13
80	24		July 31, 2.30 p. m.	5		6	6	13
81	24	July 31, 3 p. m., at dissection.	Aug. 1, 9.30 a. m.	6	18½	7	6	14
82	24		Aug. 1, 9.30 a. m.	6				
83	24	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	7	24	Aug. 8	6	15
84	24		July 31, at dissection.	5				
93	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	Aug. 8	6	14
94	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	8	6	14
95	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	8	6	14
97	25	Aug. 1, 9.30 a. m.	Aug. 2, 9.30 a. m.	6	24	8	6	14
101	26	Aug. 2, 9 a. m.	Aug. 3, 10.30 a. m.	6	25½	9	6	14
109	26	Aug. 4, 2 p. m.	Aug. 6, 10 a. m.	9	44	13	7	18
110	26	Aug. 2, 9.30 a. m.				9		14
111	27	Aug. 4, 9 a. m.	Aug. 5, 12 m.	7	27	12	7	16
116	27	Aug. 3, 6 p. m.	Aug. 4, 2 p. m.	6	20	11	7	15
123	27	Aug. 4, 9 a. m.	Aug. 5, 12 m.	7	27	13	8	17
124	27	Aug. 3, 10.30 a. m.	Aug. 4, 9 a. m.	6	22½	11	7	15
125	27	Aug. 3, 6 p. m.	Aug. 4, 9 a. m.	6	15	11	7	15
132	28	Aug. 3, 6 p. m.	Aug. 4, 9 a. m.	5	15	11	7	14
137	28	Aug. 4, 9 a. m.	Aug. 4, 2 p. m.	5	5	12	8	15
138	28	Aug. 6, 10 a. m.	Aug. 10, 10 a. m.	11	96	15	5	18
149	30	Aug. 6, 10 a. m.	Aug. 6, 5.30 p. m.	5	7½	13	7	14

[a] The data in the foregoing table may be summarized as follows: Average length of larval period for 30 larvae, 6.17 days; variation in length of larval period, 4 to 11 days; average for 23 prepupae, 24.99 hours; variation of prepupal period, 5 to 96 hours; average for 27 pupae. 6.3 days; variation for pupal period, 5 to 8 days; average for 28 adults, 14.46 days; variation period from egg to adult, 11 to 18 days.

[b] Larval stage obtained by subtracting an incubation period of two days from time elapsing between date of oviposition and date of pupation.

PREPUPA

When the larva becomes full grown and is ready to pupate it voids the meconium and reverses itself within the puparium. Turning its head in the opposite direction from the host remains and the meconial substance, it then transforms to the prepupal stage. In this stage a constriction appears between the thoracic and the abdominal segments.

The length of the prepupal stage was from 5 to 96 hours, the average being about 25 hours for 23 prepupae (Table I).

PUPA

The pupa (fig. 1, *e*) when first formed is pure white. As the pupa ages the eyes become red and the mandibles brown. After this the whole pupa very rapidly assumes a light-brown color. The pupa is from 1.5 to 1.95 mm. in length and from 0.5 to 0.65 mm. in width. The average length of 10 pupae was 1.8 mm. and the average width was 0.6 mm.

The pupal stage lasts for a period of from 5 to 8 days. The average length of the pupal stage for 27 pupae was 6.3 days (Table I). All the data on this stage are based on observations confined to pupae of the male sex.

The total period from oviposition to the emergence of the adult was from 11 to 18 days in length. The average for 28 adults was about 14½ days (Table I).

ADULT

The adult female (fig. 2, *a*) is about 2 mm. long. It may be readily distinguished from the other Hessian fly parasites by a large, faint, subcircular, fuscous spot in the middle of the fore-

FIG. 2.—*Polyscelis modestus: a*, Adult female (actual length 2.3 mm.); *b*, antenna of female; *c*, antenna of male; *d*, median leg of male, showing femur, tibia, and tarsus; *e*, wings of male. All greatly enlarged.

wing. The head and the thorax are bronzy to brassy green. The clypeal region, the under side of the thorax, and the propodeum are tinged with blue. The antenna (fig. 2, *b*) is black, except the scape which is testaceous with a fuscous apex. The legs are a reddish testaceous, except the coxae, which are concolorous with the thorax, and the apical tarsal joints, which are dark brown. The abdomen is black with bronzy metallic reflections.

The male is somewhat smaller than the female and quite different in habitus. The head and thorax are bright metallic blue-green. The antenna (fig. 2, *c*) is fuscous, except the scape, which is very pale testaceous. The legs, including all of the coxae, are pale testaceous, except the middle tibiae (fig. 2, *d*), which are black with narrow, pale testaceous bands at the base, and the apical tarsal joints, which are black. The fuscous spot is absent in the forewing of the male (fig. 2, *e*) but this sex of *Polyscelis modestus* may be distinguished from the other male Hessian fly parasites by the coloring of the legs.

LONGEVITY

The life of the adult female parasites was from 7 to 39 days in length, the average length of life for five females being about 24 days. The life of the male was considerably shorter than that of the female. It was from 5 to 11 days in length, the average for four males being about 9 days, or less than one-half of that of the average female. (See Table II.)

SEX RATIO

Thirteen adults of this species have been reared from Hessian fly puparia collected in the field. Of these 13 specimens 6 were males, 6 were females, and the sex of the remaining 1 was unrecorded, thus indicating a sex ratio of approximately 50–50.

PARTHENOGENESIS

Experimental rearings have shown that it is possible for this species to reproduce parthenogenetically. In two cases in which unfertilized females were provided with unparasitized Hessian-fly puparia for oviposition, progeny resulted which were of the male sex.

OVIPOSITION

According to observations recorded during these laboratory experiments, the first oviposition occurred from 5 to 7 days after the emergence of the adults. It is very probable, however, that under natural conditions oviposition begins somewhat earlier.

It is also presumed that under natural conditions not more than one egg is deposited within a puparium by the same individual parasite; but in the course of these experiments it was not uncommon to find two or three eggs or more than one parasitic larva within the same puparium when dissected. This is undoubtedly an abnormal habit which the parasite is compelled to assume on account of close confinement and the limited number of puparia

TABLE II.—*Longevity of the adults of Polyscelis modestus*

Emerged	Died	Longevity
FEMALES		
		Days
May 8	May 15	7
July 11	Aug. 6	26
Aug. 8	Sept. 16	39
Aug. 8	Sept. 16	39
Sept. 13	Sept. 21	8
Average		23.8
MALES		
May 7	May 15	8
Aug. 1	Aug. 6	5
Sept. 10	Sept. 21	11
Sept. 5	Sept. 16	11
Average		8.75

provided each time for oviposition. In no case did more than one adult emerge from a puparium, although in possibly 10 or more cases more than one egg or more than one larva were found within a puparium.

EFFECT OF PARASITE ON HOST

In its attack upon the host the larva of *Polyscelis modestus* liquefies the contents of the Hessian fly larva as do other chalcidoid larvae of the Hessian fly parasites which have been studied. This process of liquefaction takes place rapidly, being almost completed in 24 hours after the parasite has attacked its host. A period of quiescence follows the attachment of the parasitic larva to its host and probably it is during this period that a digestive fluid which causes disintegration is injected by the primary parasitic larva. When the internal organs of the host are completely disintegrated, the liquefied contents are rapidly consumed by the parasite.

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SUMMARY

Polyscelis modestus Gahan, which was first discovered in 1915, has thus far proved to be a Hessian fly parasite of only minor importance.

Its present known distribution is confined to the southeastern and south central parts of Pennsylvania and the north central part of Maryland.

It attacks and destroys both the larvae and the pupae of the Hessian fly, as also the larvae and probably the eggs and pupae of its own species. It is occasionally hyperparasitic, probably on *Platygaster vernalis* (Myers).

Females reproducing parthenogenetically are arrhenotokous.

Before consumption by the parasite the internal contents of the host undergo a process of liquefaction, which probably is caused by the injection of a secretion by the primary parasitic larva.

LONGEVITY AND FECUNDITY OF BRUCHUS QUADRIMACULATUS FAB. AS INFLUENCED BY DIFFERENT FOODS [1]

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INTRODUCTION

It is remarkable how writers have refrained from making definite statements in regard to the feeding habits of adult weevils, *Bruchus quadrimaculatus*. The general assumption is that no food is required, and that as soon as the energy stored up by the larva is exhausted the adult dies. This is borne out by the fact that weevils of both sexes are smaller at the end of life than at the time of emergence; and the female shrinks to about one-third her former size. In warm weather this energy is used up more rapidly than in cooler weather. The insect therefore lives longer in a low temperature than in a high one, but does not produce more eggs during the long period of life than during the shorter period at the higher temperature.

The few known statements regarding the feeding habits of this weevil are not altogether in accord with one another. In speaking of *Bruchus chinensis*, which, he says, is typical of the other bruchids, Kunhikannan (*1*)[2] says: "The adults appear to take no food during their life." Wade (*5*), speaking of *B. quadrimaculatus*, says: "Weevils were bred in successive generations, * * * the adults taking neither food nor drink throughout their existence without apparent injury to them or effect upon their activities. Under these conditions adult females were kept alive for as many as 40 days." He says further: "That the adults will drink was demonstrated on several occasions when water was given them, of which they partook greedily. So far as could be learned, access to water did not stimulate their activities or prolong their life. That they feed can not be stated definitely; none were ever observed to do so, although it is quite possible that they may feed some on the green pods and foliage of the host plant." Sanborn (*4*), discussing the same weevil, says: "The adults are not as ravenous as the young or larvæ." Paddock and Reinhard (*3*) say: "The active feeding period is confined to the larval stage." In the same publication they say: "The cowpea weevil [the common name which they apply to *B. quadrimaculatus*] has not been observed to feed on any solid food in the adult stage * * *. In the field the adults feed almost exclusively upon nectar secreted by the nectaries located at the base of the green pods."

In confinement the length of life of the adult varies from a few days to a few weeks, depending on the temperature. In a warehouse the weevil gets no food, but finds conditions favorable for reproduction during the short time it lives. This is not the case in the field. There is a long period every summer during which there is no suitable place for oviposition in the field, but the crops become infested later. This would indicate one of three things: First, that infestation is caused by weevils which have emerged during the summer shortly before the crop is sufficiently matured for oviposition to take place; or, second, that the female weevil has the ability in the field to assimilate material which should normally go to form part of the quota of eggs, as is claimed to be the case with poultry, thus being sustained until she finds a suitable place to deposit the remainder of the eggs; or, third, that she consumed some kind of nourishment which prolongs her life. Experiments and observations indicate

[1] Received for publication July 26, 1924. Issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 305.

that in the great majority of instances the first is true. The second does not appear plausible, but the third may offer a solution for very light infestations in some cases and forms the basis for the experiments herein described.

SCOPE OF THE EXPERIMENTS

The present paper gives a summary of results of experiments conducted by the writers in 1923 at Alhambra, Calif. Four different sets of experiments beginning May 7 were carried on, three simultaneously and one later. Red Ripper cowpeas in glass vials 3 inches by ⅞ inch in size, provided with cork stoppers, were used throughout the experiments.

In experiment one, 100 pairs of newly emerged weevils were placed in vials, each of which contained 6 cowpeas. The vials were numbered consecutively from 1 to 100. Into each of the vials numbered 1, 5, 9, and every fourth vial to and including 97, was put a drop of water. Into each vial numbered 2, 6, 10, and every fourth vial to and including 98, was put a drop of honey. Vials, 3, 7, 11, etc., to and including 99, were each given a drop of saturated sugar water, while the remaining vials, 4, 8, 12, etc., contained only cowpeas and weevils, used as controls. All were examined daily, the cowpeas being replaced by others, the eggs recorded, and the water, sugar water, and honey being replenished as needed. Because of evaporation the water needed to be replenished daily and the honey and sugar solution less frequently. The date when the male or female died was also recorded.

Experiments two and three were similar in arrangement except that in experiment two only one male was placed in each vial, while in experiment three only one female was placed in each vial. Some of the females were known to have mated and others had had an opportunity to mate.

In experiment four only 24 females were taken, none of which had had an opportunity to mate. They were numbered and given food in the same manner as the others, there being only six individuals in each of the groups having water, honey, sugar water, or nothing, while in each of the other experiments there were 25 in each group.

The experiments were intended primarily to determine whether or not the presence of certain foods would aid the adult weevils to live longer than they ordinarily lived under normal warehouse conditions; in other words, to try to determine if food would support these short-lived insects while their host plants were becoming sufficiently developed for oviposition to occur. Whether adult weevils will live 3 months or less than 20 days is a vital question when trying to control them in the field. Together with the length of life as influenced by different kinds of foods this summary deals with the length of life as it is influenced by mating and reproduction, the number of eggs and their viability as influenced by the food and age of the parent female, and the asexual production of eggs. These data are related to the rate of increase as well as to the control of the weevils. It is thought that they will contribute something toward a more thorough knowledge of a very vital part of the life of this destructive and widespread species.

While the principal food of *Bruchus quadrimaculatus* in California is the black-eyed cowpea, it apparently breeds as freely in any other variety of cowpeas (*Vigna sinesis*) which may be available. Because the eggs can be seen more readily on Red Ripper cowpeas than on Black-eyes, the former were used throughout the experiments.

RESULTS OF EXPERIMENT 1

Table I presents the results of experiment 1. An examination shows readily that there was not only a great difference between the average length of life of the weevils receiving liquid and that of those receiving none, but it also shows that there was a corresponding difference in the number of eggs laid. The males and females without food lived an average of 17.48 and 17.72 days, respectively, with maxima of 22 and 27 days. Those given water lived an average of 10 days longer, or 27.24 days, with a maximum of 35 days for the males and 51 for the females. Those fed on honey averaged 27.76 days, or only a fraction of a day longer than those given water, but the maximum age for males was 60 and for females 44. Those fed on sugar water lived an average of 36.84 and 30.68 days for males and females, respectively, with maxima of 56 and 54 days. For both sexes the average length of life in each group having liquid was greater than the maximum for either sex without food; while the average life of the males receiving sugar water was more than twice that of those without food. Three insects, representing both sexes, lived twice as long as any weevil with-

out food. The group of weevils which lived the shortest length of time produced the smallest number of eggs. As the total length of life in all individuals of a group increased, the total number of eggs increased. The weevils receiving sugar water produced 49 per cent more eggs than those without food.

As was stated earlier, daily records were kept of the eggs laid by each female. These daily records are summarized in Table II, which shows the total number of eggs laid each day by the weevils of each group and the number and percentage of these eggs which hatched and emerged.

That the weevils which received no food were normal for weevils so treated is borne out by numerous observations from various authors. Wade (*5*) says that in Oklahoma "The number of eggs deposited by females varies from 11 to more than 100, 75 being about the average with favorable conditions obtaining." Courtney (*3*) in Texas found that during May, 1916, the average number of eggs laid by 30 females was 81, and during June the average number for 32 females was 88 eggs. Larson and Simmons (*2*) found that during September and October the average laid by 61 females was 88.52, the minimum number being 28 and the maximum 131. Throughout these experiments only weevils were used which were known to be newly emerged and which had not obtained food or deposited eggs. They were numbered consecutively, without selection as to size, color markings, or any other character, and were given their food after being numbered. The vials in which they were contained, the cowpeas on which they deposited, and their location in the laboratory with reference to light and temperature, were uniform for all groups of weevils; any difference in the length of life of the weevils and the number of eggs produced by each group is therefore directly attributable to the difference in the foods they were given.

TABLE I.—*Length of life of males and females and number of eggs deposited by 100 pairs of Bruchus quadrimaculatus given water, honey, sugar water, and nothing*

Weevils given water				Weevils given honey				Weevils given sugar water				Weevils without food or drink			
Pair No.	Longevity of—		Eggs laid	Pair No.	Longevity of—		Eggs laid	Pair No.	Longevity of—		Eggs laid	Pair No.	Longevity of—		Eggs laid
	Males	Females			Males	Females			Males	Females			Males	Females	
	Days	*Days*			*Days*	*Days*			*Days*	*Days*			*Days*	*Days*	
1	35	18	114	2	32	10	28	3	42	9	47	4	16	18	28
5	29	21	75	6	23	23	121	7	52	[a] 3	1	8	14	17	104
9	22	45	93	10	28	10	59	11	26	29	151	12	17	12	67
13	35	45	133	14	22	22	113	15	31	22	154	16	17	12	70
17	28	22	151	18	23	26	115	19	38	54	142	20	16	14	72
21	21	21	138	22	25	36	134	23	43	27	141	24	15	15	96
25	32	11	76	26	24	27	140	27	38	[b] 17	139	28	18	18	124
29	34	27	82	30	23	23	140	31	56	35	160	32	13	13	78
33	31	45	119	34	28	34	157	35	31	34	180	36	17	20	111
37	28	20	126	38	16	44	153	39	53	21	27	40	20	17	103
41	34	24	115	42	29	33	120	43	36	41	186	44	16	17	77
45	26	19	143	46	24	24	140	47	36	33	182	48	17	27	48
49	29	22	141	50	33	19	94	51	35	27	133	52	17	22	73
53	26	28	159	54	21	33	145	55	24	26	160	56	19	16	59
57	24	22	157	58	21	34	147	59	37	19	136	60	17	10	68
61	16	16	71	62	31	38	71	63	31	33	170	64	19	22	119
65	22	23	103	66	41	26	126	67	40	31	162	68	20	20	101
69	18	26	132	70	28	38	131	71	30	41	179	72	19	19	97
73	22	23	132	74	27	31	140	75	33	36	196	76	17	19	87
77	33	51	111	78	19	22	131	79	29	47	143	80	19	15	96
81	30	20	135	82	26	29	140	83	38	42	142	84	20	20	113
85	27	19	104	86	26	33	144	87	27	40	151	88	22	20	109
89	18	40	117	90	24	43	148	91	23	45	115	92	15	22	105
93	29	48	7	94	60	18	80	95	46	39	83	96	18	22	131
97	32	25	138	98	40	18	80	99	46	16	24	100	19	16	77
Total	681	681	2,872	-----	694	694	2,997	-----	921	767	3,304	-----	437	443	2,213
Aver	27.24	27.24	114.88	-----	27.76	27.76	119.88	-----	36.84	30.68	132.16	-----	17.48	17.72	88.52
Max	35	51	159	-----	60	44	157	-----	56	54	196	-----	22	27	131

[a] Female got stuck in the sugar water.

[b] Female accidentally killed.

TABLE II.—*Number of eggs laid each day by 100 pairs of Bruchus quadrimaculatus given water, honey, sugar water, and nothing; also the number and percentage of these eggs which hatched and emerged from each group*

Day	Water					Honey				
	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged
1st	386	325	84.20	291	75.39	379	305	80.47	282	74.41
2d	330	263	79.70	228	69.09	335	275	82.09	255	76.12
3d	245	198	80.82	183	74.69	239	201	84.10	191	79.92
4th	195	159	81.54	141	72.31	179	135	75.42	126	70.39
5th	158	133	84.18	124	78.48	149	106	71.14	94	63.08
6th	163	136	83.44	127	77.91	156	101	64.74	95	60.90
7th	180	137	76.11	127	70.56	165	110	66.67	105	63.64
8th	108	86	79.63	82	75.93	144	91	63.19	85	59.03
9th	131	87	66.41	85	64.89	132	88	66.67	80	60.61
10th	117	81	69.23	77	65.81	114	66	57.89	62	54.39
11th	114	86	75.44	79	69.30	113	72	63.72	65	57.52
12th	114	82	71.93	73	64.04	103	65	63.11	58	56.31
13th	114	73	64.04	66	57.89	106	72	67.92	64	60.38
14th	109	77	70.64	65	59.63	104	71	68.27	63	60.58
15th	119	67	56.30	62	52.10	107	60	56.07	52	48.60
16th	84	31	36.90	25	29.76	102	63	61.76	55	53.92
17th	77	21	27.27	20	25.97	61	29	47.54	20	32.79
18th	47	10	21.28	7	14.89	63	34	53.97	23	36.51
19th	32	7	21.87	6	18.75	48	19	39.58	12	25.00
20th	21	1	4.76	1	4.76	46	23	50.00	18	39.13
21st	5	0				41	18	43.90	14	34.15
22d	5	0				28	11	39.29	6	21.43
23d	2	0				17	3	17.65	3	17.65
24th	0					17	9	52.94	5	29.41
25th	2	0				11	4	36.36	2	18.18
26th	1	0				8	2	25.00	1	12.50
27th	1	0				8	1	12.50	0	
28th	3	0				6	0			
29th	1	0				3	0			
30th	1	0				5	0			
31st	2	0				1	0			
32d	1	0				0				
33d	0					5	0			
34th	0					0				
35th	1	0				0				
36th	0					1	0			
37th	1	0				0				
38th	0					0				
39th	2	0				0				
40th	0					1	0			
41st	0					0				
42d	0					0				
43d	0					0				
44th	0					[a]0				
51st	[a]0									
54th										
Total or per cent	2,872	2,060	71.73	1,869	65.08	2,997	2,034	67.87	1,836	61.26

[a] Last 0 indicates date when last female died.

TABLE II.—*Number of eggs laid each day by 100 pairs of Bruchus quadrimaculatus given water, honey, sugar water, and nothing; also the number and percentage of these eggs which hatched and emerged from each group*—Continued

Day	Sugar water					Nothing				
	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged	Number of eggs	Number hatched	Per cent hatched	Number emerged	Per cent emerged
1st	313	264	84.34	237	75.72	417	327	78.42	294	70.50
2d	314	256	81.53	246	78.34	310	257	82.90	246	79.35
3d	221	172	77.83	164	74.21	225	164	72.89	147	65.33
4th	200	148	74.00	137	68.50	183	130	71.04	125	68.31
5th	153	115	75.16	109	71.24	149	119	79.87	107	71.81
6th	164	132	80.49	119	72.56	147	113	76.87	108	73.47
7th	143	121	84.62	115	80.42	132	106	80.30	101	76.52
8th	112	87	77.68	85	75.89	112	86	76.79	81	72.32
9th	126	97	76.98	94	74.60	85	57	67.06	52	61.18
10th	118	89	75.42	85	72.03	91	61	67.03	58	63.74
11th	116	83	71.55	73	62.93	89	58	65.17	51	57.30
12th	116	87	75.00	78	67.24	63	35	55.56	32	50.79
13th	129	94	72.87	89	68.99	66	47	71.21	46	69.70
14th	122	88	72.13	77	63.11	49	25	51.02	23	46.94
15th	125	93	74.40	81	64.80	47	27	57.45	25	53.19
16th	137	95	69.34	83	60.58	22	11	50.00	11	50.00
17th	102	59	57.84	51	50.00	12	4	33.33	4	33.33
18th	90	52	57.78	42	46.67	6	4	66.67	2	33.33
19th	79	45	56.96	38	48.10	4	0			
20th	70	34	48.57	30	42.86	0				
21st	58	31	53.45	25	43.10	2	0			
22d	44	20	45.45	18	40.91	2	0			
23d	45	21	46.67	18	40.00	0				
24th	22	9	40.91	5	22.73	0				
25th	33	12	36.36	7	21.21	0				
26th	24	6	25.00	4	16.67	0				
27th	19	1	5.26	1	5.26	0				
28th	16	3	18.75	2	12.50					
29th	12	2	16.67	1	8.33					
30th	12	0								
31st	10	0								
32d	3	2	66.67	2	66.67					
33d	30	5	16.67	3	10.00					
34th	9	0								
35th	4	2	50.00	2	50.00					
36th	6	0								
37th	2	0								
38th	2	0								
39th	0									
40th	0									
41st	0									
42d	2	0								
43d	1	0								
44th	0									
51st	0									
54th	[a]0									
Total or per cent	3,304	2,325	70.37	2,121	64.19	2,313	1,631	73.70	1,513	68.37

The weevils receiving water, honey, and sugar water laid 30, 35, and 49 per cent more eggs, respectively, than were laid by those which received nothing. From these eggs only 24, 21, and 40 per cent more weevils emerged than from those laid by the control group. There was a greater percentage of emergence from the eggs laid by the weevils which received no food than from those laid by either of the other groups, while the total emergence from each of the groups receiving food was considerably larger than that from the group receiving none. This difference in the percentage of emergence may be accounted for in two ways. First, it may have been accidental, because frequently when the eggs were being removed from the containers they would become smeared with sugar water, honey, or water. The probability that the eggs would become smeared was greatest in the case of the weevils which were given honey and least in the case of those given water. The percentage of emergence was lowest (67.87) for the former and highest (71.72) for the latter of the three groups fed. Second, the lower viability of the eggs laid late in the lives of the parent weevils may have caused the difference. A survey of Table II shows that the period of oviposition of the groups receiving food was much longer than that of the control group. It also shows that not only the number of eggs decreased toward the latter end of the weevil's life, but the viability also decreased. This is corroborated by Larson and Simmons' (*2*) record of 61 pairs.

Lack of space prevents the presentation of the records of individual weevils but these records show that of those without food 16 laid their maximum number of eggs on the first day, 7 on the second day, 1 on more than one day, and 1 on the fourth day. Of those given sugar water 10 laid the maximum number on the first day, 8 on the second, 2 on the third, 1 each on the fourth and sixth days, and 3 on more than one day. This tendency is carried through the groups, and accounts for the rapid and regular decrease in the number of eggs (Table II) for the group without food and the relatively slow and irregular decrease in the group receiving sugar. It is interesting to note that the total number of eggs of each group dropped to less than 100 on the sixteenth, seventeenth, eighteenth, and ninth days for those given water, honey, sugar water, and nothing, respectively. The maximum number of eggs laid by one weevil during one day was 34—laid by a female without food. While we have frequently noted higher individual records than 34, it was 8 more than the maximum for any weevil receiving sugar water.

It is of economic importance as well as of scientific interest that the last eggs were laid on the thirty-ninth, fortieth, forty-third, and twenty-second days, respectively, and that the last eggs which produced weevils were laid on the twentieth, twenty-sixth, thirty-fifth, and eighteenth days by the weevils fed on water, honey, sugar water, and nothing, respectively. It appears, therefore, that weevils receiving sugar water will deposit viable eggs during twice as long a period of time as will weevils receiving no food.

The various foods apparently had no injurious or beneficial effect on the progeny. The emergence was uniform for all groups, the first emergence from eggs laid by each group being recorded in 53 days, or on June 29. The last emergence from the water-fed group occurred on July 31, while the date of last emergence from the sugar-fed group was August 2, the date of last emergence for the other groups being August 1. Thus the last emergence occurred 34 days later than the first, and 87 days after the first eggs were laid. The sexes were about equally divided, the 1,514 emerged weevils from the group not fed being 783 males and 731 females; the 1,870 from the water-fed group were 926 and 944, the 1,836 from the honey-fed group were 919 and 917, and those from the sugar-fed group were 1,077 males and 1,044 females; thus of the 7,341 emerged weevils 50.47 per cent were males.

RESULTS WITH 100 LONE MALES AND 100 LONE FEMALES

Because these weevils are polygamous and copulate freely it is of economic importance to know what, if any, effect the state of celibacy has on the length of life of the different sexes. Table III summarizes the length of life of 100 lone male weevils which were fed in the same manner as were the 100 pairs, and of the 100 males of the 100 pairs considered in connection with Table II.

TABLE III.—*Longevity of 100 lone males and 100 mating males given water, honey, sugar water, and nothing*

Food given	Longevity of lone males			Longevity of mating males		
	Maximum	Minimum	Average	Maximum	Minimum	Average
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
Water	54	3	33.6	35	16	27.24
Honey	83	22	54.4	60	16	27.76
Sugar water	88	21	58.08	56	23	36.84
Nothing	48	20	30.96	22	13	17.48
Average			44.26			27.33

Table III shows that for both these groups of males, those which received no food lived the shortest average length of time, followed in order by those given water, honey, and sugar water. The maximum number of days lived by any individual of each group is in nearly the same ascending order. In each group the average length of time lived as well as the maximum for an individual is greater with the lone males than with the others. A lone male lived a maximum of 88 days while the maximum for a mating male was only 60 days. The average for all the unmated males was 44.27, compared with 27.33 for the other group.

The lone males and the lone females in Tables III and IV were not separated until some of them were known to have mated; in some instances, however, single individuals of each sex had emerged in separate containers. As it is known that these weevils normally copulate frequently this experiment demonstrated that a single mating was all that was necessary to fertilize the eggs laid during a long period of time. Of the group of lone females 92 per cent laid eggs. Only 73 per cent laid viable eggs. In order to determine whether the females were able to produce eggs without fertilization or whether a much larger percentage of the females had been fertilized than was believed to have been the case, another series of females, each individual of which had emerged alone in a container, was carried on. (Table V.)

Table IV summarizes the length of life of 100 lone females and the females from the 100 pairs discussed in connection with Table II, following the same method as that of Table III.

TABLE IV.—*Longevity of 100 lone females and 100 mating females given water, honey, sugar water, and nothing*

Food given	Longevity of lone females			Longevity of mating females		
	Maximum	Minimum	Average	Maximum	Minimum	Average
	Days	*Days*	*Days*	*Days*	*Days*	*Days*
Water	52	25	38.16	51	11	27.24
Honey	65	8	41.4	44	10	27.36
Sugar water	80	15	53.56	54	[a] 3	30.68
Nothing	50	18	27.8	27	10	17.72
Average			40.23			25.75

[a] Weevil got stuck in sugar water.

Table IV, following the general results of Tables I and III, shows that the average length of life of all females, lone, or mating, is shortest for the group receiving nothing, and increases for the groups receiving water, honey, and sugar water. For the lone females as with the lone males the maximum length of time lived by an individual of each group was shortest without food, longer when given water, still longer when receiving honey, and longest when fed sugar water. The lone females which received no food, although they produced an average of 62 eggs, lived an average of 10 days longer than the average number of days lived by the mating females. The lone females given water produced an average of 57 eggs and lived an average of 11 days longer than the corresponding mating females. Females receiving honey produced an average of 66 eggs and lived an average of 14 days longer than the mating females receiving the same food. The females receiving sugar water laid an average of 56 eggs each and lived an average of 23 days longer than the average for the corresponding mating females. Only in the group of lone females fed on honey did a weevil which had laid fertile eggs live as long as virgins in the same group. The maximum number of eggs laid by lone females were for nothing, water, honey, and sugar water, respectively, 110, 137, 116, and 148. For the average and maximum number of eggs laid by the mating females see Table I.

While the great majority of the viable eggs laid by the lone females were deposited early in the life of the females, some weevils emerged from eggs laid by the water-fed and honey-fed weevils when they were 24 days old. Weevils emerged from eggs laid by the sugar-water group and the group without food when they were 23 days and 18 days old, respectively.

RESULTS WITH VIRGIN FEMALES

The 24 virgin females studied in experiment 4 emerged July 16 in separate containers where no males emerged, and so had no chance to mate. Table V shows that the virgin females which did not receive food were unable to live as long as those receiving food. The virgin females lived longer than the mating females receiving the same treatment as to food (Table IV), but not as long as the lone females. The temperature during late July and August was much higher than earlier in the season, and the increased temperature undoubtedly shortened the lives of the last group of weevils. Although 17 eggs were laid by these weevils, they all failed to hatch, which would indicate that weevils of this species may deposit a small number of eggs without copulation having taken place, but that the eggs will not hatch.

TABLE V.—*Results of feeding experiments with 24 virgin females, Bruchus quadrimaculatus, fed on water, honey, sugar water, and nothing, 1923*

Fed on water			Fed on honey			Fed on sugar water			Not fed		
Number of weevils	Longevity	Eggs deposited	Number of weevils	Longevity	Eggs deposited	Number of weevils	Longevity	Eggs deposited	Number of weevils	Longevity	Eggs deposited
	Days			*Days*			*Days*			*Days*	
1	49	1	2	33	0	3	28	5	4	24	1
5	22	1	6	42	1	7	32	0	8	22	0
9	39	0	10	30	0	11	49	0	12	26	0
13	23	0	14	29	1	15	25	0	16	22	0
17	42	0	18	22	0	19	51	0	20	23	1
21	32	0	22	45	5	23	67	0	24	24	1
Total	207	2		201	7		252	5		141	3
Average	34.5	0.33		33.5	1.17		42.0	0.83		23.5	0.50
Maximum	49	1		45	5		67	5		26	1
Minimum	22	0		22	0		25	0		22	0

CONCLUSIONS

The knowledge that the average length of life of *Bruchus quadrimaculatus* is prolonged considerably in confinement by access to water and much more by access to sugar water suggests that the same species may be able to find a more suitable food in the field. Whether this food consists of only small particles of dew on the leaves, of nectar in the blossoms, of so-called honeydew on the leaves and stems, or of some other substance, remains to be learned. However, there is a decided difference between the problem of controlling weevils if they live less than 20 days on an average and 30 days as a maximum and the corresponding problem if they live an average of 50 or 60 days with 88 or more as a maximum. These problems will be discussed in another paper.

Weevils in their normal condition in the warehouse live a shorter time without food than when they have access to water or sweetened water.

The difference in the average length of life of the weevils receiving no food and those receiving water varied from less than 3 days with the lone males to 11 with the lone females. Access to water lengthened the lives of the pairs about 10 days. Sugar water lengthened their lives from 13 to 27 days as an average for different groups of weevils.

Access to water increases the average number of eggs laid by about 30 per cent, and access to sugar water increases the number of eggs about 50 per cent.

Food reduced the number of eggs laid during the first few days of oviposition, but lengthened the time over which eggs were laid. Viable eggs were laid over twice as great a period of time by weevils receiving sugar water as by those without food.

Frequent mating such as occurs normally under storage conditions reduced the length of life of both males and females. Females which mated only once during the first few hours after emergence deposited large numbers of fertile eggs.

Virgins deposited only small numbers of infertile eggs and in no case produced fertile eggs.

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A DOMINANT LETHAL CHLOROPHYLL MUTATION IN MAIZE [1]

By J. H. KEMPTON

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There are two outstanding facts with respect to the large number of heritable variations found in maize: First, practically all are degenerative with a lower survival value than normal plants, and second, they usually are recessive, being carried from generation to generation in latent form. These two facts bear a direct relation to each other, for if deleterious variations were not recessive they would be eliminated promptly from the stock at a rate proportional to their lower survival value. It seems clear, therefore, that the large number of recessive as compared with dominant variations is due largely to the fact that the recessive variations represent the accumulation of mutations through many generations, while the only dominant mutations found are those which have originated recently or which have no selective value. There is, of course, a gradual elimination of the recessive mutations also, the rate depending upon their productivity in a homozygous form, but this elimination is comparatively slow.

Recently, the writer has had an excellent illustration of the elimination of a dominant mutation. In a population of 50 plants of an F_1 between two varieties of maize, one of which had been inbred for eleven, the other seven, generations there appeared a single plant one-half of which was normal green in color, the other half being yellow, of a shade similar to the yellow seedling of Lindstrom.[2]

Neither parent of the cross from which this plant arose ever has produced yellow seedlings, and none of the sister plants showed the slightest tendency in this direction. The plant as a whole was a perfect example of a sectorial chimera. The chimeral nature appeared with the first leaf and extended throughout the entire plant. One-half of each leaf as well as one-half of the tassel was yellow, the affected portion being on the same side of the plant in each case. This division was apparent even on the central spike, where all the spikelets on one side were yellow and those on the other green. Two upper tassel branches were similar to the central spike, but the other branches of the tassel were divided equally, being either all green or all yellow. In appearance, the plant resembled the chimera reported by Khadilkar [3] except that in the case he reported the affected half was variegated yellow and white instead of self-yellow.

The plant was very weak and when mature was less than one-third the size of its normal sibs. The growth also was slower and the flowers matured about 10 days later than those of the normal plants. No ear was formed but pollen was produced abundantly.

Pollen was collected separately from the self-yellow and the self-green branches and as self-fertilization was not possible the pollen from the two sorts of spikelets was applied to two sister plants in an unrelated progeny which had been self-fertilized for 13 generations and which had never produced either albino or yellow seedlings.

Being inbred for such a long period, the resulting ears were not as large as could be desired, but 200 seedlings were grown from each, those having the green section of the chimera as a male parent giving only green seedlings while those with the yellow section of the chimera as a male parent gave 102 green and 98 yellow seedlings. This obviously is an approximation of the 1 : 1 ratio expected if the yellow portion of the plant was heterozygous for a dominant factor for the yellow chlorophyll disorder.

Most of the seedlings were grown out of doors in the ordinary manner but a few seeds were reserved for planting in greenhouse flats to provide a comparison with seedlings raised from the self-pollinated green sibs which survived in the field culture.

[1] Received for publication July 29, 1924 Issued January, 1925.

[2] LINDSTROM, E. W.—CHLOROPHYLL INHERITANCE IN MAIZE. N. Y. Cornell Agr. Exp. Sta. Mem. 13, 68 p., illus. 1918.

[3] KHADILKAR, T. R.—A SECTORIAL CHIMERA IN MAIZE. Jour. Heredity 12: 284–285. 1921.

The yellow seedlings were similar to the normal green seedlings in all respects except color. Those grown out-of-doors died after producing three or four leaves while the green plants grew normally without indication of chlorophyll disorders. Fifteen of these surviving green plants together with 18 of the plants having the green section of the chimera as a male parent were self-pollinated.

From these self-pollinated ears 4,489 seedlings were grown in the greenhouse, all being normal with no recurrence of the yellow character. In addition, the progeny of self-pollinated normal sibs of the chimera resulted in 547 plants all normal with respect to chlorophyll disorders.

Although these greenhouse seedlings were normal with respect to the yellow coloration they exhibited another type of chlorophyll disorder. This latter form, in extreme cases, resembled ordinary albinism but with imperceptible gradation from pure white to normal green. There was much variability between the progenies of different ears in this respect, some having 100 per cent of the plants affected, while in others less than 10 per cent showed the character to a noticeable degree. Except in the most extreme cases the plants recovered after the first four or five leaves developed and then remained normal.

It was thought at first that this second deficiency was related to the dominant yellow disorder indicating a general breakdown of chlorophyll, but subsequently unrelated progenies grown under the same conditions exhibited the phenomenon. Remnants of the ears planted in the field showed no evidence of this albinistic character, and it now seems probable that this character depends for its expression on some of the peculiar environmental factors of greenhouse culture.

At the time the seedlings were grown in the greenhouse the remainder of the F_1 hybrid seeds having the yellow section of the chimera as the male parent were planted, giving 12 green and 16 yellow seedlings. Contrary to their behavior in the field, the yellow seedlings raised in the greenhouse grew slowly and finally flowered, and although no seeds were produced, a small amount of pollen was shed. The growth of these seedlings had been so slow, however, that all the normal plants had matured long before the yellow plants flowered and only plants of the annual teosinte (*Euchlaena mexicana*) were available for crossing. Several crosses were made between yellow plants and this teosinte but only 40 seeds resulted. Of these 10 seedlings are now growing, 6 being yellow and 4 green. From this behavior it seems clear that the yellow half of the chimera was the result of a dominant mutation from green to yellow in one chromosome at a very early somatic cell division since it affected almost exactly one-half of the plant as well as the germ plasm.

Since plants lacking chlorophyll can not survive, the production of a further generation of this mutation was made possible only by the fortuitous circumstance that it involved but one-half of the plant. Had it occurred at an earlier cell division or previous to fertilization, the result would have been a single self-yellow seedling which would have perished without progeny.

As none of the yellow seedlings in the subsequent generation survived in the field, and as these were the only plants carrying this dominant mutation the character would have been automatically eliminated, precluding further study. It is believed that this is the only dominant mutation observed in maize within historic times and it furnishes evidence why such mutations have not been observed more frequently.

The fact that the chlorophyll deficiency observed in this case proved heritable raises the question as to whether similar deficiencies observed in Japonica plants would not also prove to be heritable if the pollen could be collected from the white areas.

There can be no question that on extreme Japonica plants the albino stripes often involve entire spikelets of the staminate inflorescence, an excellent illustration having been afforded in the case of a Japonica plant which produced an apogamous terminal panicle where each spikelet developed into a minature plant. Some of these minature plants were perfect albinos, others variegated, and still others, self-green. With the exception of the albinos, such plants can be propagated and it seems not unreasonable to infer that normal self-green plants could be produced from the recessive Japonica type by such means. The occurrence of albino plantlets strongly suggests that had these spikelets developed normally they would have produced pollen genetically albinistic and the occurrence of albino seedlings in the progeny of many commercial strains of Japonica may be due to this phenomenon.

SUMMARY

A sectorial chimera in which one-half of the plant was yellow, the other green, appeared in an F_1 maize hybrid. Pollen from the two halves of the plant was applied to normal green plants. The cross having the green side of the chimera as the male parent gave only green seedlings in F_1 and F_2 progeny, while the cross having as a male parent the yellow side of the chimera gave equal numbers of yellow and green plants in the F_1. The green plants gave only green F_2 and the yellow plants in the field died after producing three or four leaves. Yellow plants raised in the greenhouse reached maturity and produced pollen but no silks. The pollen from these yellow plants used on the annual teosinte, *Euchlaena mexicana*, resulted in an F_1 with approximately equal numbers of yellow and green seedlings.

It is concluded that the yellow character represents a dominant mutation, lethal under field culture. From the fact that the germ plasm proved to be of the same nature as the somatic tissue, it is suggested that the same phenomenon may be present in Japonica plants where the striping extends through the inflorescence.

THE RATE OF GROWTH OF GREEN AND ALBINO MAIZE SEEDLINGS [1]

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For several years continuous records of elongation of many species of plants growing naturally in the field have been obtained by means of an auxanometer adapted to field use.[2] Chief among the plants measured is maize and from the hundreds of records obtained for this species it has become increasingly evident that much individuality exists in the responses of plants to meteorological changes even where such plants are related closely and are growing in near proximity. These differences in reaction between individuals in many cases must result from very small environmental fluctuations, though in others it seems more probable that the observed behavior is the result of inherent differences within the plants. In general, however, the elongation of all maize plants is accelerated by increased temperature, at least below 100° F. and probably above this temperature. The minimum rate is reached shortly after the minimum temperature, usually an hour or two before sunrise. In addition, maize plants seem not to be sensitive to changes in temperature or solar radiation which persist for less than 30 minutes.

In connection with the inherent differences between plants in their response to temperature and solar radiation it became of interest to compare the elongation rate of albino seedlings with that of their normal green sibs.

As is well known, there are several forms of albino seedlings in maize, the most extreme of which show no trace of chlorophyll. The growth of such seedlings should be comparable to that made by the sprouts of stored tubers or bulbs where the elongation results from the transfer of stored material. The latter type of elongation, however, is checked by light, whereas albino seedlings growing in sunlight continue to increase in size until the endosperm is exhausted. If solar radiation is a direct factor in the elongation of maize (for which there is some evidence) albino seedlings would be expected to react very differently from normal green plants since the practically pure white leaves would reflect a large part of the light. The reflection of light might be expected to result in a lower temperature for such plants but in reality there can be little difference since the leaves of normal green plants are maintained at practically air temperature through the effects of transpirations.

There is little difference in the size of normal and albino maize seedlings until three leaves have been produced, but after this the normal plants forge rapidly ahead while the albinos as rapidly decline. It is not unusual to find albino seedlings with four leaves and more rarely with five, but for the most part the leaves above the third are very much smaller than similar leaves of normal plants. Normal plants seem to derive little benefit from the material elaborated by the first few leaves unless the size of the root system is increased, though an inspection of the roots of albino and normal seedlings of the same age reveals no outstanding differences.

The accompanying figure is one of several very similar figures obtained from sister maize seedlings grown in the same hill in the field. The graph shows the hourly elongation rate of the second leaf in each case, the air temperature, and the solar radiation as measured by the differential thermograph devised by Briggs.[3]

The growth curves are remarkedly alike, the greatest difference consisting of 0.2 mm., being little larger than the experimental error. It is apparent from the figure that the maximum hourly rate of elongation is no greater in the green than in the albino plants, and that, like the green plants, the albinos have their maximum rate during the

[1] Received for publication July 29, 1924. Issued January, 1925.

[2] Collins, G. N., and Kempton, J. H. A field auxanometer. Jour. Wash. Acad. Sci. 6:204–209, illus. 1916.

[3] Briggs, L. J. A mechanical differential telethermograph and some of its applications. Jour. Wash. Acad. Sci. 3: 33–35, illus. 1913.

hours of daylight. Both plants show a rather closer agreement with radiation than with air temperature.

not so obviously dissimilar show very different reactions to variations in radiation and temperature. Such be-

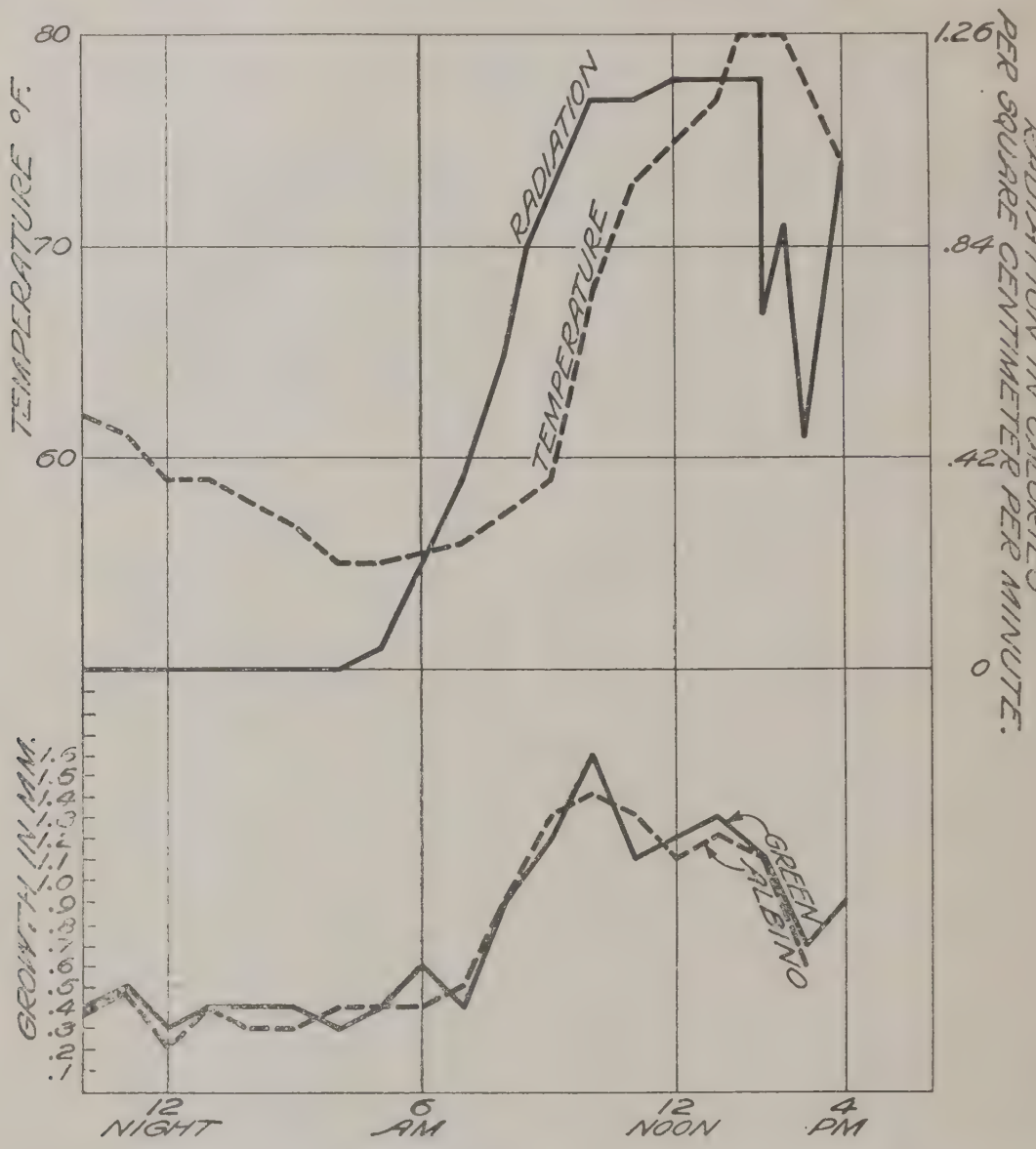

FIG. 1.—Hourly rate of growth of green and albino maize seedlings compared with air temperature and solar radiation

It seems rather remarkable that these sister maize plants differing so greatly in a major physiological characteristic should have such similar growth rates when in many other instances plants

havior shows that the response of the plant to air temperature and radiation is controlled by inherent factors not associated with the production of chlorophyll and photosynthesis.

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CRITICAL TESTS OF MISCELLANEOUS ANTHELMINTICS [1]

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INTRODUCTION

By critical tests of anthelmintics is meant tests of these drugs by their administration in definite known doses to animals, with subsequent collection for a suitable length of time of all worms passed and the post-mortem examination of the animals with the collection of all worms then present. Identification of the worms collected ante-mortem and post-mortem, and addition of the numbers of those of the same kind, then give the total number of each kind present at the beginning of the experiment. The relation of the number of each kind passed to the total number present at the beginning of the experiment is ascertained in percentages and this percentage is arbitrarily taken as the percentage of efficacy of the anthelmintic. Such critical testing has been carried on for about 10 years in the Zoological Division of the Bureau of Animal Industry, and hundreds of such experiments show that when properly interpreted the figures thus obtained are a good index of the efficacy of an anthelmintic. Interpretation is necessary, since anthelmintic efficacy varies with several factors such as:

THE DRUG.—Its amount, composition, age, solubility, concomitant effects (such as production of vomition, purgation, or constipation, etc.), mode of administration (such as in hard capsules, soft capsules, enteric-coated capsules, by stomach tube, by rectum, subcutaneously, intramuscularly, intravenously, intratracheally, etc.).

THE OPERATOR.—His accuracy, technic, familiarity with the animal's anatomy, skill in handling animals, etc.

THE EXPERIMENT ANIMAL.—Its special physiology, physical condition, age, peculiar conditions or existing lesions or other pathological conditions, such as atonic and distended stomachs, the complicated stomach of ruminants, etc.

CONCOMITANT PROCEDURE IN TREATMENT.—Fasting, diet, omission of purgatives, use of different purgatives, etc.

THE WORMS.—Number, size, relatively inaccessible locations like the cecum, occurrence in cysts or nodules or in the mucosa or under mucus or hemorrhagic coverings, occurrence of larvae and of adults with protective cuticles, etc.

Like many scientific investigations, these studies consist in correlating two or more variables; the number here is many more than two. Failures in anthelmintic medication may often be explained by careful consideration of these factors; and modifying those factors capable of modification may convert failure into success.

TESTS OF A COMBINATION OF CARBON TETRACHLORIDE, CHENOPODIUM, AND ARECOLINE HYDROBROMIDE FOR WORMS IN DOGS

PROTOCOLS

Dog No. 568; 7 kg.; 2.1 cc. of mixture of 3 parts carbon tetrachloride and 1 part oil of chenopodium by volume, with one-fourth grain of arecoline hydrobromide; animal died within 24 hours; post-mortem, extensive cirrhosis of liver and presence of *Dirofilaria immitis* in the heart; no worms passed and none in digestive tract, hence no conclusions as to efficacy of the treatment. *Dirofilaria*

[1] Received for publication Apr. 19, 1924—issued January, 1925.

immitis is so rare in dogs in the vicinity of Washington, D. C., that probably the few cases found are those of dogs from the southern part of the United States.

Dog No. 548; 6.5 kg.; 1.95 cc. of above mixture with one-eighth grain of arecoline hydrobromide; no worms passed in 4 days; post-mortem, on fourth day, 1 ascarid. Combination entirely ineffective against ascarids.

Dog No. 559; 7 kg.; 2.1 cc. of above mixture with one-fourth grain arecoline hydrobromide; 8 ascarids passed the first day; no worms the next three days; post-mortem, on fourth day, no worms. Combination 100 per cent effective against ascarids.

Dog No. 569; 8.5 kg.; 2.55 cc. of above mixture with one-fourth grain arecoline hydrobromide; no worms passed in four days; post-mortem, on fourth day, 98 whipworms. Combination entirely ineffective against whipworms.

Dog No. 564; 13 kg.; 3.9 cc. of above mixture; first day, 2 whipworms; second day, 25 whipworms; third day, 3 whipworms; fourth day, negative; post-mortem, on fourth day, 32 whipworms. Combination 48 per cent effective against whipworms.

Dog No. 591; 16.5 kg.; 4.95 cc. of above mixture with one-half grain arecoline hydrobromide; first day, 1 hookworm; second and third days negative; fourth day, 112 whipworms; post-mortem, on fourth day, 17 whipworms. Combination 100 per cent effective against hookworms and 87 per cent effective against whipworms.

Dog No. 592; 14.5 kg.; 4.35 cc. of above mixture with one-half grain arecoline hydrobromide; animal appeared sick in a few minutes and died in a few hours. No post-mortem examination and no conclusions.

Dog No. 593; 14.5 kg.; 4.35 cc. of above mixture with one-half grain of arecoline hydrobromide; no worms passed in 4 days; post-mortem, on fourth day, 14 whipworms. Combination entirely ineffective against whipworms.

Dog No. 594; 14 kg.; 4.2 cc. of above mixture with one-half grain of arecoline hydrobromide; first day, 1 hookworm; second, third, and fourth days negative; post-mortem on fourth day negative. Combination 100 per cent effective against hookworms.

Dog No. 595; 6 kg.; 1.8 cc. of above mixture with one-fourth grain arecoline hydrobromide; first day, 2 hookworms; 128 whipworms; second, third, and fourth days negative; post-mortem on fourth day negative. Combination 100 per cent effective against hookworms and whipworms.

Dog No. 596; 13 kg.; 4.2 cc. of above mixture with one-half grain of arecoline hydrobromide; first day, 3 hookworms, 2 whipworms; second day, 1 whipworm; third and fourth days negative; post-mortem, on fourth day, 1 whipworm. Combination 100 per cent effective against hookworms and 75 per cent effective against whipworms.

Dog No. 600; 10 kg.; 3 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day negative; second day, 2 hookworms; third and fourth days negative; post-mortem, on fourth day, 25 hookworms, 1 whipworm, 6 tapeworms. Combination 7 per cent effective against hookworms and entirely ineffective against whipworms and tapeworms.

Dog No. 601; 13 kg.; 3.9 cc. of above mixture with one-half grain arecoline hydrobromide; first day, 1 hookworm; second, third, and fourth days negative; post-mortem, on fourth day, 19 whipworms. Combination 100 per cent effective against hookworms, entirely ineffective against whipworms.

Dog No. 602; 10 kg.; 3 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day, 1 hookworm; second, third, and fourth days negative; post-mortem, on fourth day, 18 whipworms. Combination 100 per cent effective against hookworms, entirely ineffective against whipworms.

Dog No. 603; 7 kg.; 2.1 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day, 6 hookworms; second day, 1 whipworm; third and fourth days negative; post-mortem, on fourth day, 1 whipworm. Combination 100 per cent effective against hookworms and 50 per cent effective against whipworms.

Dog No. 604; 9 kg.; 2.7 cc. of above mixture with one-fourth grain of arecoline hydrobromide; no worms passed in 4 days; post-mortem on fourth day, negative. No conclusions.

Dog No. 605; 5 kg.; 1.5 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day, 1 ascarid, 5 hookworms; second, third, and fourth days negative; post-mortem, on fourth day, 1 hookworm, 1 whipworm, 64 tapeworms (*Dipylidium* sp.). Combination 100 per cent effective against ascarids, 83 per cent effective against hookworms, entirely ineffective against whipworms and tapeworms.

DISCUSSION

It has been shown experimentally by Hall and Foster (*13*)[2] and by Hall in a number of subsequent papers that chenopodium at the rate of 0.1 cc. per kilogram of weight of animal is a quite dependable anthelmintic for removing ascarids from dogs and that when given with an ounce of castor oil it removes all ascarids present in almost all cases. Chenopodium is also fairly effective in removing hookworms from dogs. It has also been shown by Hall (*18*) and subsequently by Hall and Shillinger (*24*) that carbon tetrachloride at the rate of 0.3 cc. per kilogram of weight of animal is a quite dependable anthelmintic for removing hookworms from dogs, and this has been confirmed in extensive use in veterinary practice for the past 2 or 3 years. The work of Allen (*2*) and of Hanson and Van Volkenburg (*26*) shows that the same is true for hookworms in foxes. Carbon tetrachloride is also fairly effective in in removing ascarids. It has also been shown by Hall and Shillinger (*23*) that arecoline hydrobromide, first proposed as a taeniacide for dogs by Lentz (*32*), removes all tapeworms present in the majority of cases, though it fails to remove some or all in a rather large minority of cases, a thing not uncommon with tapeworm remedies. Theoretically, a combination of carbon tetrachloride, chenopodium, and arecoline hydrobromide should make a good anthelmintic for removing the ascarids, hookworms, and tapeworms from dogs and might prove a useful "shotgun" prescription under conditions preventing fecal examination to determine the sort of worms present. Hall (*18*) found the combination of carbon tetrachloride and chenopodium at a dose rate of 0.3 cc. per kilogram of weight of animal, as given here, entirely effective in removing hookworms and ascarids from dogs. The arecoline hydrobromide should provide the necessary purgation for the chenopodium and carbon tetrachloride.

The fact that the combination removed all the ascarids from 2 of 3 infested dogs, giving what may be termed cures in 67 per cent of cases; failed entirely in one case, or 33 per cent of the cases; and removed 90 per cent of the total of 10 worms present, indicates that there is little loss of efficacy of chenopodium and carbon tetrachloride against ascarids when given with arecoline hydrobromide. That a single ascarid might be missed occasionally is more or less to be expected.

It removed all the hookworms from 7 of 9 infested dogs, giving cures in 78 per cent of the cases; removed 83 per cent of the worms in one case and 7 per cent in another, giving partial cures in 22 per cent of the cases; and removed 46 per cent of the total of 48 worms present. These facts indicate that carbon tetrachloride and chenopodium, when given with arecoline hydrobromide, show a distinct loss of efficacy and dependability against hookworms.

It removed all the whipworms from 1 of 11 infested dogs, giving cures in 9 per cent of the cases; removed no worms in 6 cases, or 55 per cent of the cases; removed 87, 75, 50, and 48 per cent of the worms in 4 cases, giving partial cures in 36 per cent of the cases; and removed 57 per cent of the total of 476 worms present. This is not a bad showing, and is perhaps better than that of the individual drugs used in the combination, although this is not easy to ascertain owing to the erratic action of drugs against whipworms.

Whipworms are not good subjects for ordinary anthelmintic tests, as the efficacy of a drug appears to depend on the accident of the entry of the drug into the cecum, rather than on the effect of the drug on the worms with which it comes in contact.

It removed none of the 70 tapeworms present in two cases, giving complete failures with these worms. The striking thing about the experiments is this total failure of the arecoline hydrobromide against tapeworms. The authors have shown in a previous paper (*23*) that all the tapeworms were removed from 4 of 7 animals treated and none from 3 animals, so it is evident that dependable action can not be expected in nearly all cases. Unfortunately, the series of available animals included too few with tapeworms to make this a good test, but it certainly gives little reason for expecting much in the way of tapeworm removal from this combination.

In general, this combination does not give in test the results that would be expected from a theoretical consideration. It would probably maintain a rather high efficacy against ascarids, show a decided decrease of efficacy against hookworms and tapeworms, and at least average efficacy against whipworms, as compared with its constituents, but could hardly be recommended as a useful combination for cases where fecal examinations

[2] Reference is made by number (italic) to "Literature cited," pp. 331–332.

were not made to ascertain the kinds of worms present. It would appear to be better practice in such cases to give the carbon tetrachloride or the mixture of carbon tetrachloride and chenopodium for ascarids and hookworms and such whipworms as might incidentally be removed, and a week or two later to give arecoline hydrobromide.

The deaths of 2 animals out of 17 within 24 hours after treatment can not be definitely correlated with the administration of the drugs as the sole factor or even the primary factor, but it suggests that the treatment is none too safe and that in routine practice it would be injurious to an unduly high percentage of animals treated.

TESTS OF BENZYL-PHENOL FOR REMOVING WORMS FROM DOGS

PROTOCOLS

Dog No. 606; 8 kg; 20 grains in hard capsules; first day negative; second day, 3 whipworms; third and fourth days, negative; post-mortem, on fourth day, 61 whipworms, 11 hookworms, 3 tapeworms. Benzyl-phenol about 5 per cent effective against whipworms; entirely ineffective against hookworms and tapeworms.

Dog No. 607; 12 kg.; 20 grains one day and 30 grains the following day, given in hard capsules; no worms in 3 days after first dose; post-mortem, on third day, 5 whipworms, 3 hookworms, and 3 tapeworms. Wholly ineffective against whipworms, hookworms, and tapeworms.

Dog. No. 611; 14.5 kg.; 30 grains in hard capsules; first day, 3 hookworms; second, third and fourth days negative; post-mortem, fourth day, 9 hookworms, 6 whipworms. Effective 25 per cent against hookworms; entirely ineffective against whipworms.

Dog No. 608; 6.5 kg.; 20 grains in hard capsules; no worms in 4 days; post-mortem, on fourth day, 15 hookworms, 15 whipworms, and 3 tapeworms. Entirely ineffective against hookworms, whipworms, and tapeworms.

Benzyl-phenol in doses of 20 and 30 grains in single dose and in doses of 20 grains followed by 30 grains the following day removed three of 41 hookworms, or 7 per cent; 3 of 90 whipworms, or 3 per cent; and 1 of 9 tapeworms.

DISCUSSION

The anthelmintic value of phenols has been investigated to some extent in this country and abroad, and various phenols, such as coal-tar creosote, have been used empirically for years.

Stiles (*41*) reported that coal-tar creosote (the phenol content not given) would kill stomach worms in sheep in some cases at least and gave good clinical results in sheep, calves and grown cattle in a number of cases.

Hall and Foster (*13*) tested certain coal-tar creosote preparations on four sheep, with unsatisfactory results. Two sheep died the day after treatment and only four nodular worms were recovered from the manure of all animals. The post-mortem findings were inconclusive, but failed to show the treatments as of value. Hall and Foster also tested a coal-tar creosote preparation on two dogs. One dog passed 2 ascarids and on post-mortem examination had 3 ascarids and 3 hookworms. The other dog passed a tapeworm without the head, but as the dog had ascarids, hookworms and tapeworms, killing the animal for post-mortem examination did not seem worth while.

Caius and Mhaskar (*6*) reported tests of propenyl phenols on hookworms in man, from which they concluded that these phenols have well-marked anthelmintic properties which are not associated with the unsaturated side chain but with the phenol group. In other papers they state that the efficacy of thymol and betanaphthol is correlated with the free phenolic-hydroxyl group in these compounds.

In this connection it may be recalled that the phenols are a group of organic compounds composed of hydroxy derivatives of the benzene series, the hydroxyl radical being linked directly to the nucleus. The refined phenols include phenol, cresol and the higher phenols. In benzyl-phenol, benzoic acid, which is also a benzene ring derivative, is linked to the benzene nucleus of phenol, leaving the free phenolic hydroxyl group present as in thymol and betanaphthol. The protocols of the present investigation show that in spite of the presence of this phenolic hydroxyl group, benzyl-phenol has only about 5 per cent efficacy against hookworms, which may be regarded as the only suitable test worms present in these animals. With the doses used, a moderate amount of inflammation was present in the small intestine of three animals, and an extensive serous infiltration in the lower part of the small intestine and the cecum of the other. Dr. A. R. Albright states that benzyl phenol is

less toxic and less irritant to the mucosa than thymol and is better tolerated in the stomach. Such facts would favor this substance for use in place of thymol provided the anthelmintic efficacy were as high as that of thymol or higher. In experiments reported by Hall and Foster (*13*), thymol in doses of 4.5 to 26 grains administered in single and repeated doses to 9 animals removed 23 of 151 hookworms, or 15 per cent, the maximum efficacy, 50 per cent, being with doses of about 2 grains per kilogram (or 20 grains for an average-sized dog) repeated three times. With these repeated treatments, 1 dog passed 89 per cent of its hookworms and the others passed none. Apparently neither thymol nor benzyl-phenol is very effective against hookworms in dogs, although the doses used, approximately 2 grains per kilogram, or 1 grain per pound, are those recommended for thymol for removing hookworms from dogs. Both of these drugs appear much inferior to carbon tetrachloride for this purpose.

TESTS OF ETHYLENE DICHLORIDE FOR WORMS IN DOGS

PROTOCOLS

Dog No. 617; 9.5 kg.; 2.85 cc. in hard capsules; first day, 2 hookworms; no worms the next 3 days; post-mortem, on fourth day, 21 hookworms, 47 whipworms, 94 tapeworms. Drug 9 per cent effective against hookworms; entirely ineffective against whipworms and tapeworms.

Dog No. 645; 7 kg.; 3.75 cc. in hard capsules; no worms in 4 days; post-mortem, on fourth day, 3 hookworms, 6 whipworms, 5 tapeworms. Drug entirely ineffective against hookworms, whipworms, and tapeworms.

Dog No. 638; 14 kg.; 7 cc. by stomach tube; 1 hookworm and 1 whipworm the second day; no other worms in 4 days; same amount then given in hard capsules; first day, 11 hookworms; second day, 2 whipworms; no worms the next 2 days; post-mortem, on fourth day after second treatment, 6 hookworms, 91 whipworms, 4 tapeworms. Two treatments 67 per cent effective against hookworms, 3 per cent effective against whipworms, entirely ineffective against tapeworms.

DISCUSSION

Chloroform has been more or less used in human medicine against hookworms and has been especially recommended by Alessandrini (*1*). Schultz (*39*) found it effective in removing hookworms from dogs. Hall and Foster (*13*) found it more effective in single dose for removing hookworms than other drugs tested by them and found its combination with chenopodium quite effective, as did Hall (*16*) in later experiments. The findings with chloroform, $CHCl_3$, led Hall (*18*) to test carbon tetrachloride, CCl_4, against hookworms, this drug being found more effective against hookworm in dogs than any other drug yet known. It is of interest in this connection to note that Caius and Mhaskar (*4*) found chloroform very effective in removing hookworms from man, and a year later (*5*) stated in connection with the efficacy of this drug: "This raises the question whether a narcotic, less toxic to the host than chloroform, assisted by a purgative, would not prove a very efficient remedy for the removal of hookworms." The prophecy was fulfilled in the discovery of the value of carbon tetrachloride, Hall's first paper (*18*) on this appearing the same month (April) as that of Caius and Mhaskar. Caius and Mhaskar later carried on tests of carbon tetrachloride, confirming its value against hookworms in man, and in a summary (*7*) they state that the value of carbon tetrachloride is correlated with the cumulative effect of the halogen atoms. On this basis ethylene dichloride, $C_2H_4Cl_2$, should rank somewhere below chloroform as an anthelmintic. In the present experiments this chemical in single doses of 0.3 to 0.5 cc. per kilogram removed from 0 to 9 per cent of the hookworms; and in two doses of 0.5 cc. per kilogram removed 67 per cent. These findings apparently sustain the theoretical likelihood that this ethane derivative with two atoms of chlorine is less effective than the methane derivative, chloroform, with three atoms, and this in turn is less effective than the methane derivative, carbon tetrachloride, with 4 atoms of chlorine. The presence of hydrogen seems to increase the solubility of the chemical, carbon tetrachloride being soluble 1 part in 1,250 of water and chloroform 1 part in 161 of water; the solubility of ethylene dichloride is unknown to us but is probably closer to that of chloroform than to that of carbon tetrachloride. With the increased solubility there is an increase in undesirable systemic effects on the host animal, and a diminished efficacy against worm parasites. It may be concluded that ethylene dichloride shows a slight efficacy against hookworms in dogs, as would be expected from its chemical composition; but

this efficacy is distinctly less than that of carbon tetrachloride, as would also be expected since the halogen concentration, to which the anthelmintic efficacy is apparently due, is distinctly less in ethylene dichloride than in carbon tetrachloride.

TESTS OF FERROUS SULPHATE FOR REMOVING WHIPWORMS FROM DOGS

PROTOCOLS

Dog. No. 585; 8.5 kg.; 56.4 grains in 30 cc. of water with 5 grains of calomel; no worms passed in 5 days; post-mortem on fifth day, 2 whipworms, 1 hookworm, 3 tapeworms. Entirely ineffective.

Dog No. 586; 8.5 kg.; 42.3 grains in 22.5 cc. of water; no worms in 5 days; post-mortem, on fifth day, 13 whipworms. Entirely ineffective.

Dog No. 587; 11 kg.; 28.2 grains in 15 cc. of water with 5 grains of calomel; no worms in 5 days; post-mortem, on fifth day, 24 whipworms, 2 hookworms, 4 tapeworms. Entirely ineffective.

Dog No. 588; 11 kg.; 32.9 grains in 17.5 cc. of water; no worms in 5 days; post-mortem, on fifth day, 4 whipworms. Entirely ineffective.

Dog No. 589; 13 kg.; 87.8 grains in 45 cc. of water with 5 grains of calomel; first day, 1 whipworm; second day, 2 whipworms; third day, 1 whipworm; fourth and fifth days, negative; post-mortem, on fifth day, 109 whipworms. Treatment 4 per cent effective against whipworms.

Dog No. 590; 12.5 kg.; 70.5 grains in 37.5 cc. of water; no worms in 5 days; post-mortem, on fifth day, 89 whipworms and 3 tapeworms. Entirely ineffective.

DISCUSSION

It has been pointed out by Hall (*10; 12*) that even feeble anthelmintics, such as ferrous sulphate, will occasionally remove whipworms from dogs; but, as noted above (p. 315), the efficacy of a drug in removing these worms depends on its entry into the cecum. This entry might be insured by the use of a drug of low toxicity and little irritant quality that could be given in repeated doses or in massive doses, as previously noted by the authors and as stated by Lambert (*29*). For repeated doses santonin seems the most satisfactory drug known at present. For bulky doses the latex of *Ficus laurifolia*, a fig which grows in South and Central America, appears to be favored in human medicine. This has been used by physicians in the region in which it grows, and has been recommended by Berrio, Mouatt-Biggs, and others. It is given in the comparatively large doses of 10 to 45 gm., and is said to be the best drug known for administration in this way against whipworms.

Theoretically, ferrouss ulphate might prove to be of value in this connection, since its toxicity is low and its slow movement through the digestive tract might insure its entry into the cecum in a fair number of cases when given in rather large doses. The drug is often prescribed in veterinary medicine for worms in various animals, although its use is almost entirely on an empirical basis. The present experiments indicate that in doses of 28.2 to 87.7 grains to dogs weighing from 8.5 to 13 kg., it shows little efficacy against whipworms and can not be depended on to remove these worms in single doses.

TESTS OF CHENOPODIUM INTRAMUSCULARLY AND INTRAVENOUSLY FOR REMOVING WHIPWORMS FROM DOGS

PROTOCOLS

Dog No. 549; 6 kg.; 0.5 cc. chenopodium intramuscularly in large muscles of hind leg; no worms in 4 days. Treatment ineffective, as this animal showed whipworm eggs in feces.

Dog No. 552; 9 kg.; 1 cc. chenopodium intramuscularly in large muscles of hind leg; no worms in 4 days. Treatment ineffective, as this animal showed whipworm eggs in feces.

Dog No. 609; 8 kg.; 1 cc. chenopodium intravenously; no worms in 2 days; animal dead second day; post-mortem showed 6 whipworms attached in cecum, 1 whipworm unattached in colon, 12 tapeworms (*Dipylidium* sp.). Treatment comparatively ineffective, assuming, as seems probable, that the whipworm unattached in the colon would have passed out in a day or so and that the treatment should probably be credited with its removal.

Dog No. 610; 7 kg.; 0.5 cc. intravenously; 1 whipworm the second day; some tapeworm fragments, but no heads, during 4 days; post-mortem, on fourth day, 14 whipworms, 5 hookworms, and 28 tapeworms. Drug 7 per cent effective against whipworms; entirely ineffective against hookworms and tapeworms.

DISCUSSION

The idea of removing whipworms by means of intramuscular injections seems to have occurred first to Strong (*42*), who administered to human patients thymol and chenopodium, each in sterile olive oil, without results in the form of whipworms passed or noticeable diminution in the number of eggs present in the feces.

More recently Lambert (*29*) has reported tests on human patients of intramuscular and intravenous injections of chenopodium. For his intramuscular injections Lambert used in two cases a mixture of 60 cc. oil of chenopodium, 60 cc. of camphorated oil, and 4 grams of resorcin, giving 4 cc. in one case and 10 cc. in another. After the dose of 4 cc. (equivalent to 2 cc. of chenopodium), the patient passed 4 hookworms in the course of 4 days; an anthelmintic by mouth removed 89 hookworms, 1 ascarid, and 1 whipworm. After the dose of 10 cc. (equivalent to 5 cc. of chenopodium), the patient passed 2 hookworms and 1 ascarid in the course of 3 days; an anthelmintic by mouth removed 155 hookworms and 1 ascarid. After a dose of 3 cc. of undiluted chenopodium the patient passed 3 hookworms, 22 whipworms, 9 pinworms; no anthelmintic was given by mouth, but hookworm eggs persisted in the feces after treatment.

For intravenous injections Lambert used pure oil of chenopodium in doses of 2 cc. in one case and 1.5 cc. in two other cases. After the dose of 2 cc. the patient passed 11 whipworms and 882 pinworms in the course of 3 days; chenopodium given by mouth removed 33 hookworms and 1 whipworm. One patient given 1.5 cc. intravenously passed 19 whipworms and 2 ascarids in the course of 4 days; no anthelmintic was given by mouth, but fecal examination showed hookworm eggs present in the feces. The other patient given 1.5 cc. intravenously passed 30 whipworms and 2 ascarids in the course of 3 days; no anthelmintic was given by mouth, but fecal examination showed hookworm eggs present in the feces.

From Lambert's experiments it appears that chenopodium intramuscularly will remove some whipworms, ascarids, hookworms, and pinworms, being apparently rather effective against whipworms in some cases, but not promising anything vaulable against the other worms named. Intravenously chenopodium removed whipworms, ascarids, and pinworms, the efficacy against whipworms being most evident, with the possibility that this treatment is fairly effective against ascarids and pinworms, although the evidence is not sufficient to warrant the idea that it is certainly as effective as other measures known to be less dangerous.

In the present experiments intramuscular injections of chenopodium made the animals lame and failed to remove whipworms. It does not therefore appear to be a promising mode of treatment for removing whipworms from dogs. Intravenous injections apparently removed 1 whipworm from each of 2 dogs, leaving 6 and 14 worms, respectively. Judging from Lambert's results a much higher efficacy might be obtained in individual cases, but the results in these two cases do not look promising, although intravenous injections show a greater efficacy in removing whipworms than intramuscular injections show.

A low efficacy seems indicated in the case of dogs and a high efficacy in human patients, judging from the usual numbers present in man in the region involved. This method of treatment should be investigated further. Possibly drugs more satisfactory than chenopodium could be found for intravenous injection. In the human cases syncope followed the injections; and in one dog the syncope immediately after the injections was followed by general incoordination, pronounced sickness with decubitus, and death the second day.

TESTS OF NOVARSENOBENZOL IN SOLUTION BY MOUTH, SUBCUTANEOUSLY AND INTRAVENOUSLY FOR WHIPWORMS IN DOGS

PROTOCOLS

Dog No. 612; 10.5 kg.; 0.6 gm. subcutaneously; no worms in 6 days; the dog died after 11 days; too badly decomposed to warrant post-mortem examination following negative results.

Dog No. 613; 5 kg.; 0.15 gm. intravenously; no worms in 2 days; 0.75 gm. intravenously the third day; no worms in 4 days; post-mortem, on fourth day after second treatment, 19 whipworms, 9 tapeworms. Wholly ineffective.

Dog No. 615; 15 kg.; 0.45 gm. intravenously; no worms in 4 days; no post-mortem. Apparently wholly ineffective.

Dog No. 616; 13 kg.; 0.3 gm. by mouth; no worms in 4 days; after 42 days this dog (now weighing 18.5 kg.)

was given 0.6 gm. by mouth; first and second days, no worms; third day, 4 whipworms; fourth day negative; 0.6 gm. by mouth on fourth; fifth day, 2 whipworms; sixth day, 3 whipworms; seventh day negative; eighth day, 1 whipworm; 0.6 gm. by mouth on eighth day; ninth and tenth days negative; eleventh day, 1 whipworm; twelfth day, 1 whipworm; 0.6 gm. by mouth on twelfth day; fecal examination negative from thirteenth to twenty-sixth day; doses of 0.6 gm. by mouth on sixteenth, twentieth, and twenty-fifth days; post-mortem, twenty-sixth day, 9 ascarids, 14 hookworms, 25 tapeworms (*Dipylidium* sp.). Treatment 100 per cent effective against whipworms, ineffective against hookworms, ascarids, and tapeworms.

DISCUSSION

Lundsgaard (*33*) has reported that 12 days after the last of five doses of 0.3 to 0.9 gm. of neosalvarsan a human patient passed a decomposed strobila of *Taenia saginata*. The present experiments were too brief to determine whether novarsenobenzol might similarly affect dog tapeworms. They do not indicate that novarsenobenzol in single doses subcutaneously or intravenously as given is of value in removing whipworms or other worms from dogs. Theoretically, the whipworm, which has its head and anterior body usually embedded in the mucosa of the cecum, or occasionally in that of the colon, might be poisoned in feeding on blood or serum. The action of chenopodium indicates that something of the sort is possible, but apparently novarsenobenzol is not a suitable drug for the purpose.

Given by mouth in repeated doses, novarsenobenzol removed all whipworms, a total of 12, after four doses amounting to 2.1 gm., no worms being present at the time three subsequent doses for a total of 1.8 gm. were given. So far as efficacy in repeated doses is concerned, this drug is apparently capable of replacing santonin in removing whipworms. Its efficacy by mouth is apparently along the lines we have previously outlined—that whipworms are easily removable by even feeble anthelmintics provided the anthelmintic comes in contact with the worms—and such contact can be insured by the use of repeated doses of a drug of relatively low toxicity and little irritant properties, or of large doses of such a drug in cases where large doses may be used. As regards its safety, the dog dosed by mouth showed a somewhat darkened liver and some inflammation of the small intestine after a total of 3.9 gm. administered; the findings do not indicate that the drug as given is dangerous, although little can be concluded from the findings on one animal. The principal objection to its use would be its expense.

TESTS OF TARTAR EMETIC IN SOLUTION INTRAVENOUSLY FOR WHIPWORMS IN DOGS

PROTOCOLS

Dog No. 637; 6.5 kg.; 1 grain in 1 cc. of sterile distilled water; no worms on first day; animal died in 24 hours; post-mortem showed 5 whipworms, 112 tapeworms (*Dipylidium* sp.). Entirely ineffective against whipworms and tapeworms. This dog showed extensive hemorrhages of the stomach and small intestine. The liver was yellow and friable and on section by Dr. Leigh Giltner of the Pathological Division of this bureau showed passive congestion with capillary hemorrhage, together with some atrophy of the cells, perhaps resulting from pressure of hyperemia.

Dog No. 634; 6.5 kg.; 1 grain in 1 cc. of distilled water; no worms in 4 days; no post-mortem at this time owing to total inefficacy of treatment (dog showed whipworm infestation on fecal examination).

Dog No. 633; 12 kg.; 0.5 grain in 1 cc. sterile distilled water; no worms in 4 days; no post-mortem at this time owing to total inefficacy of treatment (dog showed whipworm infestation on fecal examination).

DISCUSSION

Tartar emetic is well established in human medicine as a drug which is effective against blood flukes when administered intravenously. Theoretically it might be valuable against whipworms. The present tests indicate that in single doses of 0.5 to 1 grain it is not effective, and in certain susceptible dogs it may be highly toxic in doses of 1 grain.

TESTS OF A PROPRIETARY DRUG FOR TAPEWORMS IN DOGS

PROTOCOL

Dog No. 614; 12 kg.; 3.3 cc. (a dose rate of 2 minims per pound); first day, 6 tapeworms; next 3 days negative;

post-mortem, on fourth day, 62 tapeworms, 12 hookworms, 2 whipworms. Drug 9 per cent effective against tapeworms, entirely ineffective against hookworms and whipworms.

DISCUSSION

Since discoverers of especially effective drugs have the alternative of publishing their findings for general use or marketing their products for profit, there is always the possibility that proprietary remedies may at times be found more effective than the drugs generally known and used. For this reason the writers occasionally test proprietary anthelmintics. The proprietary preparation under consideration here is advertised as a safe and effective anthelmintic for tapeworms in dogs. A test indicates that it may fail to remove more than 9 per cent of the tapeworms from a dog. This is not surprising, as drugs intended to remove tapeworms do not show in a general way the dependable efficacy of the best drugs for the removal of nematodes. Thus occasional failures to get tapeworms occur with such standard taeniacides as male fern, kamala, areca nut, arecoline hydrobromide, etc. The proprietary in question is probably effective in many cases.

CHENOPODIUM AND MAGNESIUM SULPHATE, SIMULTANEOUSLY ADMINISTERED, TESTED FOR TOXIC EFFECTS AND EFFECTS ON WORMS IN DOGS

PROTOCOLS

Dog No. 615; 16 kg.; chenopodium at the lethal dose rate, 0.6 cc. per kilogram (9.6 cc.), by stomach tube, followed immediately by 90 cc. of a saturated solution of magnesium sulphate; fecal examination negative and no worms passed in 4 days; the animal continued in good health and was not killed; animal negative on preliminary fecal examination.

Dog No. 634; 8 kg.; chenopodium at the lethal dose rate, 0.6 cc. per kilogram (4.8 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; fecal examination negative and no worms passed in 4 days; post-mortem examination on fourth day negative for worms; the only lesion was a slight inflammation in places in the small intestine.

Dog No. 635; 11 kg.; chenopodium at the lethal dose rate, 0.6 cc. per kilogram (6.6 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; dog vomited in a few minutes and died in about 12 hours; no worms passed; post-mortem showed 5 whipworms. This dog's stomach had slightly inflamed areas; the small intestine showed excessive catarrhal enteritis, and had a marked odor of chenopodium; the cecum and large intestine were normal in appearance, though there was catarrhal material in the lumen of the large intestine, possibly from the small intestine; the lungs were much congested.

Dog No. 650; 8 kg.; chenopodium in double the minimum lethal dose rate of 0.5 cc. per kilogram, or 1 cc. per kilogram (8 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; fecal examination negative, no worms passed in 4 days, none present post-mortem on fourth day; all organs apparently normal post-mortem.

Dog No. 651; 11 kg.; chenopodium at the therapeutic dose rate for ascarids, or 0.1 cc. per kilogram (1.1 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; 1 ascarid the first day; no worms the next 3 days; post-mortem, on fourth day, 1 ascarid. Efficacy against ascarids, 50 per cent.

Dog No. 636; 8 kg.; chenopodium at the therapeutic dose rate for ascarids, or 0.1 cc. per kilogram (0.8 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; 14 ascarids the first day; no worms the next 3 days; post-mortem, on fourth day, 3 whipworms, 4 tapeworms. Treatment 100 per cent effective against ascarids; entirely ineffective against whipworms and tapeworms.

Dog No. 648; 7 kg.; chenopodium at the therapeutic dose rate for ascarids, or 0.1 cc. per kilogram (0.7 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; 1 hookworm the second day; no worms the first, third and fourth days; post-mortem, on fourth day, 54 whipworms. Treatment 100 per cent effective against hookworms; entirely ineffective against whipworms.

Dog 636 was killed by the intraperitoneal injection of 150 cc. of a saturated solution of magnesium sulphate; the animal lay down, became unconscious, and died in about 10 minutes. The

post-mortem lesions due to the drug consisted in pronounced vascular injection of the abdominal blood vessels. Dog 648 was killed by the intraperitoneal injection of 20 cc. of a saturated solution of magnesium sulphate; the animal lay down, became unconscious, and died in about 15 minutes. The lesions due to the injection were similar to those in the case of dog No. 636. This method of killing dogs has been recommended, but no previous report of the post-mortem lesions has been noted.

DISCUSSION

Macht and Finesilver (*34*) have pointed out that the simultaneous administration of magnesium sulphate with other drugs diminishes or prevents the absorption of the other drugs, apparently by virtue of the well-known salt action which causes a flow of fluid from the walls of the digestive tract to the lumen and thus inhibits an absorption current in the opposite direction. It follows from their conclusions that magnesium sulphate should not be given with other drugs where absorption of these drugs with consequent systemic effects is desired. However, it would also appear that in the case of anthelmintics, and possibly of intestinal antiseptics, the simultaneous administration of magnesium sulphate would be valuable, all other things being equal, since such substances are commonly more or less toxic, their systemic effects are not desired, and it is advisable to limit their action to that on the fauna and flora of the digestive tract if possible, leaving the host relatively unaffected. The present experiments show that magnesium sulphate saved the lives of two dogs given 0.6 cc. per kilogram of chenopodium, a little more than the minimum lethal dose of 0.5 cc. per kilogram, and of one dog given double the minimum lethal dose, but failed to save one dog given 0.6 cc. per kilogram.

The question arises whether the simultaneous administration of salts with anthelmintics will lessen the efficacy of the anthelmintic. The experiments reported above show that in therapeutic doses chenopodium with magnesium sulphate removed 15 out of 16 ascarids from two infested dogs, all the hookworms (*1*) from another, and failed to remove tapeworms and whipworms as might be expected. This drug therefore maintains its anthelmintic efficacy when given simultaneously with magnesium sulphate. Hall and Shillinger (*25*) have reported tests of the effect of magnesium sulphate administered simultaneously with carbon tetrachloride, and find the latter as effective against hookworms when administered in this manner as when administered without the salts. They were unable to obtain definite results as to the diminished toxicity of carbon tetrachloride given with the salts, as dogs tolerated very large doses of carbon tetrachloride when given alone or with the salts. In human practice Lambert (*28*) finds that carbon tetrachloride is as effective when given with the salts as when given alone, and that there is less complaint of headache and dizziness after its administration in this manner. This indicates that there is an increase in the safety of carbon tetrachloride administration when given with salts.

The additional safety of anthelmintics when given with magnesium sulphate must be checked against another factor than inhibition of absorption by virtue of the osmotic action occurring in the presence of salts. This is the factor of purgation itself. Hall (*12*) has noted that, contrary to a frequently repeated statement, it is not dangerous to give male fern with castor oil. He cites experiments in which dogs given lethal doses of male fern with adequate doses of castor oil survived, whereas the same dose without the castor oil was fatal. In human medicine various physicians, such as Leichtenstern (*31*), concur in this. Hall also reports an experiment in which the lethal dose of male fern given with 6 grains of calomel was not fatal to a dog. In the same paper he reports that dogs survived the administration of dangerously high to lethal doses of chenopodium with large doses of castor oil. Thus purgation alone by means of such purgatives as castor oil or calomel is highly protective against anthelmintics, as clinical experience also indicates, and may of itself save an animal from an otherwise lethal dose of an anthelmintic. On theoretical grounds, sustained by experimental evidence, the salt action of magnesium sulphate should add to this purgative action the inhibition of absorption by virtue of pronounced osmosis with fluids entering the lumen of the digestive tract. Even with this added action, magnesium sulphate, as one of the present experiments shows, may fail occasionally to save one animal from a lethal dose, although saving another animal from a dose at a higher rate.

The further element of speed of action must be considered in selecting purgatives in connection with anthelmintics. There has been more or less

debate on the value of castor oil and magnesium sulphate in connection with chenopodium. So far as evidence is available, the results are very good when castor oil is given at the same time as chenopodium. When purgatives are not administered until 2 or 3 hours after a final dose of chenopodium, magnesium sulphate is preferable to castor oil, largely because its more rapid action is needed when a depressant drug like chenopodium has been in the digestive tract for several hours and has established a condition of stasis and constipation. Yet the action of magnesium sulphate, other than its mere rapidity in causing purgation, indicates that it is preferable to castor oil even for simultaneous administration with anthelmintics, provided no diminution of anthelmintic efficacy follows. It has been shown, as noted above, that no diminution occurs with castor oil, and the same is apparently true in regard to magnesium sulphate, in connection with chenopodium.

TESTS OF CARBON TETRACHLORIDE AND MAGNESIUM SULPHATE, SIMULTANEOUSLY ADMINISTERED, ON WORMS IN MONKEYS

A male chimpanzee, about 1.5 years old was given 3 cc. of carbon tetrachloride with an ounce of magnesium sulphate dissolved in 90 cc. of water, by stomach tube; it passed 2 hookworms and 14 nodular worms the first day; 3 nodular worms the second day; a total of 2 hookworms and 17 nodular worms.

A female chimpanzee, about 1.5 years old, given same treatment as above, passed 1 hookworm the first day, 1 whipworm the second day; a total of 1 hookworm and 1 whipworm.

These animals were treated at the request of the owner and were not killed. Although the actual efficacy of the drug as used can not be given, the experiment is recorded here since it indicates that carbon tetrachloride as given will remove hookworms and nodular worms from monkeys. There is so little information in regard to anthelmintics for use in these animals that even this item might prove useful. Toxicity tests of carbon tetrachloride on monkeys were reported by Hall (*19*), Lake (*27*) and Hall and Shillinger (*24*). Their reports show that monkeys tolerate single doses of 6 cc. per kilogram, and doses of 1 to 5 cc. for animals weighing 2.21 to 2.63 kg. repeated 12 to 16 times for totals of 16 to 66 cc. The drug removed at least 1 whipworm and a number of heterakids (*Subulura distans*), but since the animals were not killed the exact efficacy was not ascertained.

TESTS OF THE EFFICACY AND SAFETY OF CARBON TETRACHLORIDE WHEN ADMINISTERED SIMULTANEOUSLY WITH MAGNESIUM SULPHATE TO SHEEP

PROTOCOLS

Sheep No. 6c; 10 cc. carbon tetrachloride in hard capsules followed immediately by approximately 128 gm. (4 ounces) of magnesium sulphate in 250 cc. of water; first day, 96 stomach worms (*Haemonchus contortus*), 102 small trichostrongyles, 5 tapeworms with many tapeworm fragments; second day, 94 stomach worms, 72 small trichostrongyles, 1 nodular worm, a few tapeworm fragments; third day negative; fourth day, 2 stomach worms; post-mortem, on fourth day, 1 young female stomach worm embedded in coagulum, 1 whipworm, 10 tapeworms (one tapeworm mature, the others either very young or the remainders of strobila with the major posterior portions removed by the treatment). Since the coagulum in which the young stomach worm was embedded (the regular method of occurrence of young forms) presumably protected it against anthelmintic action, the treatment may be considered 100 per cent effective against stomach worms. Hall and Shillinger (*24*) have pointed out that most of the worms killed in the stomach by anthelmintics become just so much proteid material and are digested, so that probably only the worms near the pylorus escape digestion and appear in the manure as dead worms or worm fragments. The efficacy of anthelmintics against such worms is therefore higher than that indicated by a comparison of the number of worms passed and the number present post-mortem. The stomach worms present in the check animal, No. 9c, noted below, is evidence of the high efficacy of the treatment in this case and the following cases. The treatment was also 100 per cent effective in removing small trichostrongyles, indicating that the high efficacy noted in some cases in the writers' previous report is increased by the simultaneous administration of the magnesium sulphate. The efficacy against nodular worms, of which only one was found, is also 100 per cent; experience shows that it is substantially as difficult to secure 100 per cent efficacy

when only one worm is present, or a few worms, as when many are present. The efficacy against tapeworms, 33 per cent, is high for carbon tetrachloride in case of tapeworms, and suggests that the use of the magnesium sulphate has increased the efficacy of the drug against these worms. The treatment failed to remove the whipworm present. The explanation for such failures is given in the discussion of whipworms in dogs.

Sheep No. 7c; 10 cc. carbon tetrachloride in hard capsules followed immediately by approximately 128 gm. (4 ounces) of magnesium sulphate in 250 cc. of water; no worms passed in 4 days; post-mortem, on fourth day, a fragment of a dead stomach worm in stomach, 10 whipworms. The treatment was 100 per cent effective against stomach worms; the dead worm and the presence of stomach-worm eggs in the feces before treatment, together with the number of stomach worms present in the check animal, No. 9c, show that these worms were present and that all were killed and digested, no worms appearing in the feces. If small trichostrongyles were originally present they were destroyed and passed in unrecognizable condition. The treatment failed to remove whipworms.

Sheep No. 8c; 10 cc. carbon tetrachloride in hard capsules followed immediately by approximately 128 gm. (4 ounces) of magnesium sulphate in 250 cc. of water; first day, 6 nodular worms; no worms the next 3 days; post-mortem, on fourth day, negative. The treatment was entirely effective against nodular worms, and apparently entirely effective against stomach worms, as eggs of these worms were present before treatment.

Sheep No. 9c, a check animal, not treated, was examined post-mortem to determine the probable stomach-worm infestation present in the other animals before treatment. This sheep had 1,434 stomach worms, and it is probable that somewhat similar infestations were present in the three animals treated, the drug destroying all the worms present except the young one still embedded in coagulum.

DISCUSSION

Hall and Shillinger (*24*) found that doses of 8, 12, 15, 18, 24, and 48 cc. carbon tetrachloride left no stomach worms in eight sheep infested with these worms as shown by fecal examination, a check animal having 612 stomach worms. A dose of 4 cc. left 14 stomach worms, indicating that this dose was too small to remove all of the worms, although a very high efficacy, perhaps 98 per cent, is probably indicated by the findings on the check animal and what is known of the digestion of these worms when dead in the stomach, coupled with the efficacy in the other animals. In the present series of experiments, 10 cc. of carbon tetrachloride was 100 per cent effective against stomach worms.

Hall and Shillinger found doses of 4, 8, 15, and 30 cc. carbon tetrachloride were 100 per cent effective against hookworms for all infested animals, four in number. There were no hookworms present in the experiment animals reported on in the present paper, so no tests could be made along this line. Doubtless carbon tetrachloride would be quite as effective when given with magnesium sulphate as when given without it. They found that doses of 12 to 48 cc. carbon tetrachloride removed 30 per cent of the nodular worms present in one series of animals; doses of 15 to 30 cc. removed 3 per cent of the nodular worms from one animal and 100 per cent from another; and doses of 4 and 8 cc. failed to remove any nodular worms. In the present series of cases a dose of 10 cc. removed all nodular worms from two infested animals. This 100 per cent efficacy with a dose of 10 cc. may be correlated on theoretical grounds with the use of the magnesium sulphate, which would tend to keep the drug from absorption and to carry it to the large intestine where the nodular worms occur. Additional experiments should be carried out to determine whether this dose will regularly maintain so high an efficacy when given with magnesium sulphate.

Hall and Shillinger found that doses of 12 to 48 cc. of carbon tetrachloride administered to one series of sheep removed 82 per cent of 801 small trichostrongyles of the genera Nematodirus, Cooperia, Ostertagia, and Trichostrongylus. This is of considerable interest, as no anthelmintic previously studied had shown any efficacy against these worms. Doses of 15 to 30 cc. removed only 3 per cent of 1,100 trichostrongyles present in two other animals; and doses of 4 and 8 cc. failed to remove any of 121 of these worms in two others, so far as could be determined by examination of the feces. In the experiments reported in the present paper, carbon tetrachloride in a dose of 10 cc. removed all of 174 small trichostrongyles present in one animal. Possibly the magnesium sulphate loosens these worms from mucus

on the walls of the digestive tract as a result of the flow of current to the lumen, thereby enabling the carbon tetrachloride to reach and destroy them. This matter deserves further study, because some of these small trichostrongyles, such as species of Nematodirus, Cooperia, and Trichostrongylus, are known to be pathogenic and are not as yet known to be amenable to treatment by any drug other than carbon tetrachloride.

Hall and Shillinger found that carbon tetrachloride in doses of 12 to 48 cc. removed from 30 to 90 per cent of the whipworms present. In the experiments reported in the present paper, 10 cc. of carbon tetrachloride failed to remove the whipworms present.

Hall and Shillinger did not find carbon tetrachloride of value in removing tapeworms from sheep, the drug removing one worm and leaving large numbers. Its much greater value in the experiments reported in the present paper may be correlated with the use of magnesium sulphate. In personal conversation the writers have been told that magnesium sulphate alone will remove some tapeworms from sheep, although no experimental evidence along this line is available.

In the experiments reported here, carbon tetrachloride in doses of 10 cc. simultaneously administered with 4 ounces of magnesium sulphate dissolved in 250 cc. of water, proved 100 per cent effective against stomach worms, nodular worms, and small trichostrongyles. This finding confirms previous work of the writers on stomach worms, and indicates an even higher efficacy than previously reported for the drug against nodular worms and small trichostrongyles. The increased efficacy is apparently correlated with the simultaneous administration of magnesium sulphate. At present it can not be said just how safe carbon tetrachloride is for ruminants.

Carbon tetrachloride was 33 per cent effective against tapeworms, an increase over the very low efficacy previously reported by the writers against these worms; and this increase also may be correlated with the use of magnesium sulphate. It failed entirely against whipworms, only one of these being present. Such a result might be expected in many cases in spite of excellent results in individual cases, because the drug does not always enter into the cecum where these worms are.

As regards the toxicity of carbon tetrachloride for sheep, Hall and Shillinger reported that a lamb weighing 15 kg. (33 pounds) was down and apparently dying on the fourth day after receiving 30 cc. of carbon tetrachloride in 2 ounces of castor oil. This is a large dose for so small an animal, although dogs would tolerate much larger doses. As the authors have noted elsewhere, ruminants are not so tolerant of many volatile anthelmintics as are carnivores.

No bad effects were noted for the 10 cc. dose reported in the present paper, but on post-mortem examination the kidneys of No. 7c were found to present a pathological appearance, and on microscopic examination by Dr. Leigh Giltner of the Pathological Division of this bureau they showed a subacute parenchymatous nephritis with general degeneration of the epithelium. This finding suggests that some of the carbon tetrachloride may have been absorbed in spite of the presence of the magnesium sulphate. This may not be a serious matter, as the studies of Meyer and Pessôa (*35; 36*), Smillie and Pessôa (*40*), Docherty and Nicholls (*8*), and Lamson and McLean (*30*), supported by many clinical observations in human and veterinary medicine, indicate that lesions due to carbon tetrachloride of the kidneys and liver usually clear up and leave these organs in practically their original condition in a comparatively short time. The exceptions to this rule apparently occur in those cases where these organs are already damaged and unable to tolerate further insult.

LARGE-SCALE TESTS OF REPEATED DOSES OF CARBON TETRACHLORIDE FOR WORMS IN SHEEP

To obtain further information on the possibility of using carbon tetrachloride as a control measure for worms in sheep, a flock of 23 sheep was put on monthly treatments. At the first dosing in October, 1923, 17 of these sheep were given 5 cc. of carbon tetrachloride in capsule, followed immediately by 2 oz. of a saturated solution of magnesium sulphate as a drench. Two sheep died because the solution of magnesium sulphate entered their lungs and toxic action appears to followed its absorption by them. The amount of solution appears to have been too small to cause death by asphyxiation; much larger amounts of water or normal saline may be introduced into the lungs of animals without bad effects. Magnesium sulphate is known to be toxic when administered intravenously or injected

solutions by mouth in rare instances. The use of intraperitoneal solutions of magnesium sulphate to kill dogs has recently been advocated, and the writers have found it a comparatively rapid and painless method, the most striking post-mortem lesions consisting in a vascular engorgement of the blood vessels of the viscera of the abdominal cavity.

In view of the bad effects on these sheep, the remaining six were each given 6 cc. of carbon tetrachloride with two No. 10 capsules of pure magnesium sulphate. Some of these animals appeared quite uncomfortable for a few minutes after dosing and showed a rapid respiration.

In further experiments some changes were made in the number of sheep and the individuals used in this flock, and the dose adopted for routine use was 5 cc. of carbon tetrachloride in a hard capsule, No. 13, holding a little more than 5 cc., together with dry magnesium sulphate in an equivalent bulk, about 5 cc., in the same-sized capsule. This treatment has continued now for 5 months since the first treatment, a total of 6 treatments, and fecal examinations show that it is of value in controlling infestations with various worms. It is planned to continue such monthly treatment for about a year, at the end of which time post-mortem examinations, together with the tables of weights for the period of the experiment, will show something more definite as to the value of the treatment.

TEST OF ARSENIC FOR REMOVING WORMS FROM HORSES

PROTOCOL

Horse No. 233, weighing 735 lbs., was given the following preparation: Arsenic, 30 grains; calomel, 60 grains; animal charcoal, 80 grains. This was administered in a hard gelatine capsule in the morning after fasting the animal from noon of the previous day, and was followed by a quart of water administered as a drench, with no feeding until 3 hours after treatment. The manure was examined daily for the next 6 days and no worms were found. The animal was then given copper sulphate as noted in the next experiment and, as noted in that experiment, was found to have numerous worms (*Habronema* spp., *Strongylus* sp., and cylicostomes) present post-mortem. The conclusions are similar for both experiments.

DISCUSSION

Although arsenic and combinations of arsenic and copper sulphate are effective in controlling stomach worms in sheep and goats, and the combination is quite effective in controlling tapeworms, hookworms, and to some extent nodular worms in these animals, arsenic in the dose given and as administered does not appear to be promising as an anthelmintic for worms in horses, although it has long been recommended and used for this purpose. Winslow (*43*) states: "Large single doses of arsenious acid (℥ss) are sometimes given with calomel (℥i) and aloes (℥iv) in a ball to horses to kill roundworms." This is the dose of arsenic tested here. As the horse used had no ascarids, no conclusions are possible on the value of arsenic against these worms in the horse; but other tests with arsenic suggest that carbon bisulphide and carbon tetrachloride, and perhaps chenopodium, are superior to arsenic for this purpose.

TEST OF COPPER SULPHATE FOR REMOVING WORMS FROM HORSES

PROTOCOL

Horse No. 233, weighing 735 pounds, was given 8 gm. of copper sulphate in 192 cc. of distilled water, the solution being administered as a drench. This was given in the morning after fasting the animal from noon of the previous day. The horse was not fed until an hour after treatment. The manure was examined daily for the next 9 days and no worms were found. The animal was killed on the ninth day and worms collected from it as follows: Numerous specimens of *Habronema* spp.; 221 *Strongylus* sp. collected and counted and an additional 104 estimated as present, or a total of 325 *Strongylus* sp.; 122 cylicostomes collected and counted and an additional 1,088 estimated as present, or a total of 1,210 cylicostomes.

DISCUSSION

The treatment must be regarded as ineffective, although it can not be concluded that some worms of the genus Habronema might not have been destroyed by the copper sulphate, for, as pointed out above, when worms in the stomach are killed by any anthelmintic, only the worms near the pylorus may be expected to escape digestion. In the long journey through the large intestine

of the horse such small worms as Habronema may be macerated beyond recognition.

TESTS OF NOVARSENOBENZOL ON STRONGYLES IN VERMINOUS ANEURISMS OF THE HORSE

PROTOCOL

Horse No. 223, an old animal weighing 710 pounds, was given daily intravenous injections of novarsenobenzol (Billon) in amounts of 3.6 gm. dissolved in 40 cc. of sterile distilled water, slowly injected into the jugular vein. The animal showed no pronounced symptoms from the injections until the fourth day, when it became uneasy 5 minutes after the injection, staggered and almost fell, breathed hard, and passed watery feces and some gas. It tried to eat but stopped to look at its flanks and the skin quivered over the flank region. The respiration was 68 and shallow. Apparently the drug caused cramps and extreme peristalsis. The animal was killed 3 days after this last dose, and an examination was made for worms in an aneurism of the anterior mesenteric artery which was about 7 or 8 inches long and involved the nearby arterial branches. Embedded in the thrombus and in the thickened arterial walls were 14 live specimens of *Strongylus vulgaris* in a late larval stage, all ensheathed in a somewhat loose cuticle. One additional specimen could not be definitely identified since it appeared to be either a dead and partially disintegrated strongyle or a shed cuticle filled with some dark material.

In a previous paper Hall and Shillinger (*20*) have reported failure to destroy these worms with intravenous injections of tartar emetic and of carbon tetrachloride. So far no treatment is known for destroying these worms; and, on the available evidence, no promising results can be expected from tartar emetic, carbon tetrachloride, or novarsenobenzol.

GENERAL SUMMARY AND DISCUSSION

EXPERIMENTS ON DOGS

Ascarids.—A mixture of carbon tetrachloride, 3 parts by volume, and chenopodium, 1 part by volume, given at a dose rate of 0.3 cc. per kilogram with one-eighth to one-half grain arecoline hydrobromide according to the size of the dog, shows a rather high efficacy, 90 per cent, in removing ascarids from dogs, but this efficacy is slightly less than that usually obtained with chenopodium or carbon tetrachloride given without arecoline hydrobromide. The number of infested animals and of worms present in the experiment was not sufficient to warrant the conclusion that the slight loss in indicated efficacy would be sustained in a larger series of cases.

The simultaneous administration of magnesium sulphate with a therapeutic dose of chenopodium at the rate of 0.1 cc. per kilogram gives an indicated efficacy of 94 per cent, showing that the salts do not materially diminish the efficacy of the chenopodium.

Novarsenobenzol in repeated doses by mouth was entirely ineffective against ascarids.

Hookworms.—The mixture of carbon tetrachloride, chenopodium and arecoline hydrobromide removed all the hookworms from only 78 per cent of the dogs in these experiments, removing 46 per cent of the total number of hookworms present. This is a decided loss of efficacy compared to the 100 per cent efficacy of carbon tetrachloride alone or of the mixture of carbon tetrachloride and chenopodium, contraindicating the simultaneous use of arecoline hydrobromide with these drugs for this purpose.

The therapeutic dose of chenopodium for ascarids when given with salts removed the one hookworm present; no positive conclusion can safely be drawn from this test.

Benzyl-phenol in single doses of 20 and 30 grains and in doses of 20 grains one day and 30 grains the following day removed only 7 per cent of the hookworms from the experiment animals. While chemicals having a free phenolic-hydroxyl group have a certain efficacy against hookworms, as shown here, the efficacy of such compounds, including thymol, is low against hookworms in dogs.

Ethylene dichloride at a dose rate of 0.3 to 0.5 cc. per kilogram of weight of animal removed 0 to 9 per cent of the hookworms in single doses and in two doses at the rate of 0.5 cc. per kilogram removed 67 per cent from infested dogs. This efficacy is lower than that obtained by Hall and Foster (*13*) and by Hall (*15*) for chloroform, their average for single doses being 52 per cent; and is distinctly lower than the 100 per cent efficacy of carbon tetrachloride in single doses at the rate of 0.3 per kilogram. This loss of efficacy appears to be associated with the diminished

halogen content of ethylene dichloride (Cl_2) as compared with chloroform (Cl_3) and especially with carbon tetrachloride (Cl_4).

Chenopodium intravenously in a dose of 0.5 cc. failed to remove hookworms from an infested dog. In three human cases Lambert also failed to remove any hookworms from infested patients by intravenous injections of chenopodium. By intramuscular injections of chenopodium in two cases he recovered from 1.5 to 4 per cent of the indicated total of hookworms present as ascertained by subsequent treatment by mouth; a third patient passed three hookworms and was found by fecal examination still infested, but was not subsequently treated by mouth.

Ferrous sulphate in single doses by mouth removed no hookworms from dogs.

Novarsenobenzol in repeated doses by mouth removed no hookworms from one infested dog.

WHIPWORMS.—As has been repeatedly pointed out by Hall, whipworms are actually very susceptible to even quite feeble anthelmintics, in spite of the fact that it is extremely difficult to remove them with any certainty. This might be expected on theoretical grounds. The difficulty in their removal is due to the above-mentioned uncertainty of entry of anthelmintics into the cecum.

This very uncertainty, due to their location, may account for their actual susceptibility to even feeble anthelmintics. Worms in the stomach and small intestines are constantly exposed to the effects of all sorts of substances taken in with food and water or administered as drugs, and must be fairly tolerant of many substances to maintain themselves in the face of constant exposure to them. Since many of these substances are absorbed in the stomach and small intestine, and since whipworms have a protected situation in the cecum, into which only a part of the contents passing the ileocecal or ileo-colic valve ever enters, the whipworms are much less exposed to substances entering the mouth, and therefore have much less occasion to develop a tolerance for them or immunity to them. This paper reports the passage of whipworms after the administration by mouth of the combination of carbon tetrachloride, chenopodium, and arecoline hydrobromide; also of benzyl phenol and of ethylene dichloride, with no dependable efficacy shown for any of these substances.

Whipworms have been made the object of a more varied attack than have any other worms in the digestive tract, because they are so difficult to remove. The topic, recently summarized by Lambert (*29*), may be summarized further in connection with the experiments given here.

The methods of attack are as follows:

SURGICAL.—Both in human and veterinary medicine the difficulties experienced in removing these worms by anthelmintics have led to the recommendation by the physicians Berard and Vignard (*3*) and by the veterinarian Miller (*37*) that resection of the appendix in man and of the cecum in dogs be resorted to for the removal of these worms.

ORAL MEDICATION WITH REPEATED SMALL DOSES.—Hall (*11*, *14*, *17*) has recommended the use of repeated small doses of some nonirritant anthelmintic as a method of removing whipworms, and finds santonin the most satisfactory drug for this purpose in the case of dogs. He notes that Wade in human medicine has recommended the use of 2-grain doses of thymol 3 times a day over a period of 2 weeks. To date santonin has afforded fairly satisfactory results. In the experiments reported in this paper novarsenobenzol in repeated doses by mouth removed all the whipworms from an infested dog. Evidently this drug is but feebly anthelmintic, and whipworms are very susceptible to even quite weak anthelmintics, since the treatments removed all the whipworms but failed entirely against ascarids, hookworms, and tapeworms. This method of attack with repeated small doses of a nonirritant anthelmintic of relatively low toxicity is still a most promising line of attack for the removal of whipworms.

ORAL MEDICATION WITH MASSIVE DOSES.—In addition to the use of repeated small doses, one solution of the problem of getting an anthelmintic into the cecum in contact with whipworms, is the use of single massive doses of a relatively nontoxic substance. In human medicine the latex of a fig, *Ficus laurifolia*, native in South and Central America, appears to be such a drug. In the present experiments the use of iron sulphate has been tested on the assumption that it might be effective when administered in large doses. The results indicate that it is not valuable in doses of from 28.2 to 87.8 grains, since it failed to remove whipworms from five dogs and removed only 4 per cent of these worms from one dog. The fig latex referred to above has the disadvantage of not being available outside of the areas in

which the fig grows, as the latex does not stand shipment, and it also has the disadvantage of being toxic in some cases. This method of attack promises considerable usefulness if a drug of low toxicity, capable of administration in bulky doses, that will stand shipment and will keep well, can be developed.

INTRAMUSCULAR AND SUBCUTANEOUS INJECTION.—This method of attack for whipworms appears to have been tested first by Strong (*42*), who found thymol and chenopodium intramuscularly of no value with human patients. More recently Lambert (*29*) has tested chenopodium intramuscularly on human patients and recovered 22 whipworms in one case. In the present experiments this method of treatment with chenopodium gave entirely negative results on two dogs. Apparently it will remove whipworms in some cases, but the action of the drugs tested does not seem dependable. Novarsenobenzol tested subcutaneously in one case gave negative results. This appears to be the first test of subcutaneous medication for this worm. Possibly better drugs will be found and will give a dependable action warranting their use in intramuscular injections. Whipworms appear to feed on blood or serum and it is theoretically possible to poison them by drugs introduced into the system by intramuscular or intravenous injections. The experimental and clinical evidence to date sustains this possibility, but does not sustain the idea that the action is dependable for the drugs tested.

INTRAVENOUS INJECTIONS.—Intravenous injections for removing whipworms appear to have been tested for the first time by Lambert (*29*) on three human patients, all of them passing whipworms, from 11 to 30 in number. This treatment, so far as tested, cannot, however, be expected to offer dependable action on dogs. Intravenous injections removed one whipworm from each of two infested dogs, showing that the intravenous injection of an anthelmintic will remove whipworms; but after treatment 6 worms remained in one case, and 14 in the other. If the treatment were safer it would still promise much of value, but the production of syncope in the human and other cases, and of death in one dog, indicate rather definitely that chenopodium intravenously is too dangerous for use. There remains, however, the possibility of finding a safe drug which will be effective against whipworms when administered intravenously, and this deserves investigation. This possibility has been given some consideration in the experiments with novarsenobenzol and tartar emetic reported here, but negative results with five dogs indicate that these drugs do not fulfill the necessary conditions as regards efficacy.

RECTAL INJECTIONS.—For the removal of whipworms from sheep, Brumpt, as reported by Railliet (*38*), has used rectal injections of 1 to 1.5 liters of water containing a thymol emulsion, at the rate of 1 gm. thymol to each 3 to 5 kg. of weight of animal. The efficacy of this method is not certain. Hall and Shillinger (*22*) have tested the use of rectal injections of various drugs for the removal of heterakids from the ceca of chickens, and found that chenopodium in doses of 0.1 cc. in 5 cc. of a bland oil removed 90 per cent of the worms. Subsequently, Freeborn (*9*) has reported that nicotine sulphate solution, containing 40 per cent nicotine in a dilution of 1 cc. to 200 cc. of distilled water, administered in doses of 10 cc. by rectal injection, removed 85 per cent of the heterakids. The ceca of birds are much more accessible by rectal injections than is the cecum of mammals.

The foregoing shows that whipworms have been attacked by surgical measures, by single doses of the ordinary anthelmintics by mouth, by repeated small doses of non-irritant anthelmintics of relatively low toxicity, by single massive doses of anthelmintics of relatively low toxicity, by intramuscular injections, by subcutaneous injections, by intravenous injections, and by rectal injections.

TAPEWORMS.—It has been shown by Hall and Shillinger (*23*) that arecoline hydrobromide, given as recommended by Lentz (*32*), in critical tests removes all of the tapeworms from dogs in the majority of cases, and fails to remove any in a minority of cases, so far as tested on seven dogs. It is shown in this paper that when given simultaneously with therapeutic doses of a mixture of carbon tetrachloride and chenopodium, arecoline hydrobromide apparently suffers a serious loss of efficacy, failing to remove any tapeworms from two infested animals. In view of the uncertainty of action of tapeworm remedies in general, not too much may be judged from the failure here, but the combination in question fails to warrant the expectations based on the action of its individual constituents.

An extensively advertised proprietary remedy for removing tapeworms from dogs removed only 9 per cent

of these worms from one infested dog. The only conclusion warranted in this case is that this preparation will not always remove all of the tapeworms from infested dogs, but this conclusion applies to tapeworm remedies in general.

Of the other substances tested here for their effect on various worms, chenopodium and magnesium sulphate simultaneously, benzyl-phenol, ethylene dichloride, ferrous sulphate and novarsenobenzol by mouth failed to remove any tapeworms, as did chenopodium and novarsenobenzol intravenously.

INFLUENCE OF MAGNESIUM SULPHATE ON TOXICITY OF ANTHELMINTICS.—An experiment on four dogs showed that three animals survived a lethal dose of chenopodium when given with an adequate dose of magnesium sulphate, and one animal died. The survival of three animals confirms in the case of chenopodium the possible protective action of magnesium sulphate noted by Hall and Shillinger (*25*) in the case of carbon tetrachloride. At the same time it is noted that the protective action is not entirely a function of the salt action of magnesium sulphate in inhibiting absorption by virtue of the creation of a flow of fluids from the wall of the digestive tract to the lumen, because Hall has shown that purgatives like castor oil and calomel exert a protective action against lethal doses of such drugs as chenopodium and male fern. The protective action is therefore partly due to purgation alone.

EXPERIMENTS ON MONKEYS

Carbon tetrachloride given with magnesium sulphate, will remove hookworms and nodular worms from monkeys, but the exact efficacy is not known.

EXPERIMENTS ON SHEEP

The present findings confirm the previous findings of Hall and Shillinger (*25*) on the value of carbon tetrachloride in removing various worms from sheep, and indicate that the simultaneous administration of magnesium sulphate with this drug may increase its efficacy. Doses of 10 cc. of carbon tetrachloride with magnesium sulphate are apparently 100 per cent effective in removing stomach worms (*Haemonchus contortus*), nodular worms, and small trichostrongyles, and on the basis of the writers' previous work on carbon tetrachloride for hookworms in sheep, and carbon tetrachloride and magnesium sulphate for hookworms in dogs, will probably be 100 per cent effective in removing hookworms from sheep. The same dose shows an efficacy of 33 per cent in removing tapeworms from sheep, and in repeated doses would presumably serve to clear out these worms. The dose used failed to remove whipworms from sheep, as might be expected to occur in most cases for reasons given above in the discussion of whipworms in dogs.

ARSENIC.—A test of arsenic in a single dose of 30 grains indicates that as given it is of no value in removing stomach worms (*Habronema* sp.), palisade worms (*Strongylus* sp.) and cylicostomes from the horse.

COPPER SULPHATE.—Copper sulphate (8 gm.) in solution was entirely ineffective, apparently, in removing stomach worms, palisade worms, and cylicostomes from the horse.

NOVARSENOBENZOL INTRAVENOUSLY.—Daily intravenous injections of novarsenobenzol for 4 days, totaling 14.4 gm., failed to kill strongyles in the anterior mesenteric artery. Tartar emetic and carbon tetrachloride intravenously have also been previously reported by the writers as failing to kill these worms in aneurisms.

GENERAL CONCLUSIONS

A mixture of carbon tetrachloride 3 parts by volume, and chenopodium, 1 part, at the rate of 0.3 cc. per kilogram accompanied by ⅛ to ½ grain arecoline hydrobromide, a combination which should on theoretical grounds be valuable against ascarids, hookworms and tapeworms in dogs, was found to maintain the rather high efficacy of 90 per cent against ascarids, but was considerably less efficacious against hookworms as compared with its constituents without the arecoline hydrobromide, removing only 46 per cent; and it failed entirely to remove tapeworms. The arecoline hydrobromide appears to diminish the efficacy of the other drugs against hookworms, possibly by its very rapid purgative action, purgation commonly occurring in from half an hour to an hour, and, so far as can be judged from two cases, the efficacy of the arecoline hydrobromide against tapeworms suffers a dimunition from the presence of the two other drugs.

Benzyl-phenol proved only slightly anthelmintic for hookworms, removing only about 5 per cent of the hookworms present.

Ethylene dichloride proved only slightly effective against hookworms when given at a dose rate equivalent to the therapeutic dose rate for carbon tetrachloride.

Ferrous sulphate in large doses showed very little efficacy against whipworms and is evidently not dependably effective in single doses for removing these worms.

Chenopodium intramuscularly failed to remove whipworms from two infested dogs. Intravenously it removed only one whipworm from each of two infested dogs, showing that the drug has some anthelmintic action when thus given, as Lambert had found, but also showing a lack of dependable action even in dangerous doses.

Novarsenobenzol subcutaneously failed to remove any whipworms from the one dog on which the drug was thus tested. Intravenously in single dose to one dog and in two doses to another it failed to remove any whipworms.

Tartar emetic in single dose intravenously to three dogs failed to remove any whipworms.

A proprietary remedy for tapeworm in dogs removed only 9 per cent of the tapeworms present.

Magnesium sulphate simultaneously administered with lethal doses of chenopodium to dogs protected them from the toxic effects of the chenopodium, three out of four animals surviving the dose. Hall has shown that castor oil or calomel will protect dogs against the toxic effects of lethal doses of such drugs as chenopodium and male fern, from which it appears that the protective action of magnesium sulphate is due not only to its salt action but also to its purely purgative action. Our experiments also show that magnesium sulphate simultaneously administered with therapeutic doses of chenopodium does not diminish the efficacy of the chenopodium against ascarids, and suggest that the same is true as regards effect on hookworms. The writers have previously shown that magnesium sulphate simultaneously administered with carbon tetrachloride does not diminish the efficacy of this drug against hookworms.

Carbon tetrachloride, given with magnesium sulphate, will remove hookworms and nodular worms from monkeys, but the exact efficacy is unknown.

Carbon tetrachloride given to sheep in doses of 10 cc. followed immediately by 128 gm. of magnesium sulphate shows 100 per cent efficacy against stomach worms, nodular worms, and small trichostrongyles. Previous evidence from other experiments indicates that it is also 100 per cent effective against hookworms. When thus administered with magnesium sulphate there is a marked increase in efficacy against tapeworms, this treatment removing 33 per cent of these worms instead of being almost entirely ineffective. A flock of sheep is now being treated with monthly doses of 5 cc. of carbon tetrachloride and an equivalent bulk of dry magnesium sulphate to determine the value of such repeated treatments in controlling worms in sheep.

Arsenic in the 30-grain dose ordinarily recommended for worms in horses apparently failed entirely to remove any stomach worms, palisade worms, or cylicostomes from a horse. Copper sulphate (8 gm.) in solution apparently failed entirely to remove any stomach worms, palisade worms, or cylicostomes from a horse.

Novarsenobenzol intravenously for 4 days for a total amount of 14.4 gm. failed to kill strongyles (*Strongylus vulgaris*) in a verminous aneurism in a horse.

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STUDIES ON THE INHERITANCE OF EARLINESS IN WHEAT [1]

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INTRODUCTION

The studies reported in this paper were undertaken primarily to determine the inheritance of earliness in wheat in connection with breeding for earlier varieties. As the cross under observation was intended purely for economic purposes, only general notes were taken on the F_1 progeny. But in regard to the F_2 and F_3 progenies, earliness notes were taken on individual plants and families.

The investigation of the problem of early maturity in plants has engaged for many years the active interest of geneticists and plant breeders. The value of such investigation has been emphasized by the practical importance of developing varieties of crop plants suitable for the early market or of such precocity as to escape unfavorable conditions induced by climate or crop pests.

In general, the behavior of the hybrid material in the different studies reported has been similar. In the F_1 progeny, time of heading or maturity has been found to be intermediate between that of the parents, with the means or the hybrid inclining toward one or the other parent. In the F_2 the tendency has been for the intermediate condition to be maintained but with a range of variation almost covering the extremes of both parents, and both with and without the grouping into classes showing definite segregation. In subsequent generations the segregation into distinct classes becomes more and more marked as homozygosity is approached.

REVIEW OF LITERATURE ON INHERITANCE OF EARLINESS

EXPERIMENTS WITH WHEAT

William Farrer (*6*)[3] early observed that the progeny in the F_1 generation was intermediate between the parents, and that there was no difference in this respect in the reciprocal of a cross. In the F_2 he found rather wide variation in time of ripening.

Biffen (*2*) found that the F_1 produced ripe grains well along toward the date of ripening of the late parent of an interspecific cross between an early (Polish) and a late (Rivet) wheat. In the F_2, although all plants flowered simultaneously, many plants ripened at the same time as the early parent, while some were almost ripe and still others green. He concluded that earliness is dominant.

Fruwirth (*8, p. 176, 238, 314*) believes the early ripening to be partially dominant in wheat rye, and barley.

Thompson (*15*) made numerous crosses between eight varieties of wheat, ranging from very early to late maturing. In ten F_1 crosses with the late parent the ripening period of most plants was near the mean of the late parent. In the F_2 most individuals were intermediate, with no indication of "heaping up" except in the intermediate position, but the range of variation extends nearly from that of the earliest to that of the latest of the parent varieties. He found, as did Biffen (*2*), that earliness and lateness are inherited independently of other characters. He also made the interesting observation that the earliest plants of an F_2 population frequently were quite as large and productive as the latest grandparent.

Freeman (*7*) made crosses between durum and common white wheats, as well as between two common wheats. He found that the average date of first heading in both F_2 and F_3 progenies in every case was intermediate but nearer to that of the late parent. In the durum cross, there was an extension of range much beyond that of the late parent.

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[3] Reference is made by number (italic) to "Literature cited," p. 347.

In a cross between Sonora and Turkey wheats, Bryan and Pressley (*3*) found the F_1 progeny almost exactly intermediate between the parents in time of first heading. In the F_2 generation the range of variation was but slightly greater than that of both parents but inclined toward the late parent. In the F_3 progeny several early plants were isolated which were almost as early as the early parent. In the F_4 progeny, the range of heading dates of a somewhat larger number of early plants was considerably narrower than that of the early parent.

EXPERIMENTS WITH OTHER CROPS

In studies of earliness in peas, Mendel (*1, p. 337*), found that the time of flowering of the F_1 generation was intermediate between those of the parents. Keeble and Pellew (*11*), in their work with time of flowering and stature of peas, concluded that lateness was dominant over earliness and that apparently there is a relation between the morphological and vegetative characters and the period of flowering in plants.

Leake (*12*), in studying the blooming time of cotton, found the F_1 generation intermediate, while the F_2 progeny formed a regular curve with the mean nearest the late parent.

Emerson and East (*5*) found that the time of first exposure of the anthers was distinctly intermediate in the F_1 generation of a cross between a dent corn (late) × pop corn (early). In the F_2 progeny some of the plants were nearly as early as the earliest of the early parent and others as late as the late parent. Ten families held the same relative order of ripening as of flowering, while the parent varieties were further apart in ripening than in blossoming.

Hoshino (*10*), working with rice, found that emergence of the head in the F_1 generation inclined to that of the early parent. In the F_2 progeny he found two segregation groups, with the minimum frequency midway between the variation ranges of the parents, and with the early group the larger. Further segregation groups appeared in the F_3 and F_4 generations. He explains his results by a 3-factor hypothesis.

Caporn (*4*), in reporting on the inheritance of earliness in an oat cross, found the F_1 progeny intermediate between the parents in ripening. In the F_2 progeny much variation was observed, but in the F_3 generation the variation did not extend so far as that of the parents.

INDICES OF EARLINESS IN CEREAL CROPS

The indices used in the studies on the inheritance of earliness in the cereal crops have varied considerably. Farrer (*6*) took as his index the change in color of the peduncle immediately below the spike. Biffen (*2*) used the fully ripe condition, as indicated by yellow straw and glumes and hard grain, although in order to get a comparable reading of his populations he also used half ripe, as indicated by yellow awns, other parts yellowish green, and the grain soft. His third class was green throughout. Thompson (*15*) used the term "fully ripe" for the time when the central kernels of the spike reach the dough stage. Caporn (*4*) in studies on oats considered fully ripe to indicate disappearance of the last trace of greenness from the tips of the paleae.

Freeman (*7*) used as the index of earliness in wheat the date of appearance of the first head on each plant, as did also Bryan and Pressley (*3*). Hoshino (*10*), in rice, used the shooting time, i. e., the appearance of the first spikelet above the sheath of the inflorescence. Emerson and East (*5*) used the time of first exposure of the anthers in corn.

In comparing earliness of varieties of cereals in agronomic experiments, the progression of the variety toward maturity usually is designated by several different stages. In wheat these generally include first heading, fully headed, first ripe, and fully ripe. There is usually quite close correlation between these different stages in the development of the plants. Then, in all but the exceptional cases, the stage that will be most sensitive, that is, that will show the greatest divergence in time of appearance, should most accurately show the relative earliness of varieties or strains. It is well known, and also has been the writer's experience in agronomic note-taking for a number of years, that the relative difference in earliness at first heading is considerably greater than at maturity. The rate of development of the wheat plant is more rapid at the time of ripening than at the time of heading. Ripening often is more or less abnormal also, owing to the effect of high temperatures, drought, etc.

Harlan (*9, p. 8, 9*), found in his studies on barley that the evidence seemed all in favor of the first emergence of the awn as a character possessing heritability equal to that of most plant characters. He also states that the date of ripening is subject to influences induced by climatic factors. In awned varieties of wheat

or barley the first appearance of the awn is a more definite stage than the first appearance of the spike. In awnless varieties emergence of the tip of the spike is practically as definite as the first appearance of the awn in an awned variety.

AGRONOMIC NOTES ON EARLINESS IN WHEAT

In the studies herein reported, curves have been prepared from the data on heading and ripening of varieties of wheat in the wheat classification nursery at Davis, Calif., in the year 1922, and also from Chico, Calif., in 1920, for comparison. Both seasons were normal, except at Chico where the temperature was relatively high during the period of ripening. Figure 1 shows the curves of first heading, all headed, first ripe, and all ripe for 29 representative varieties for the Davis nursery in 1922.

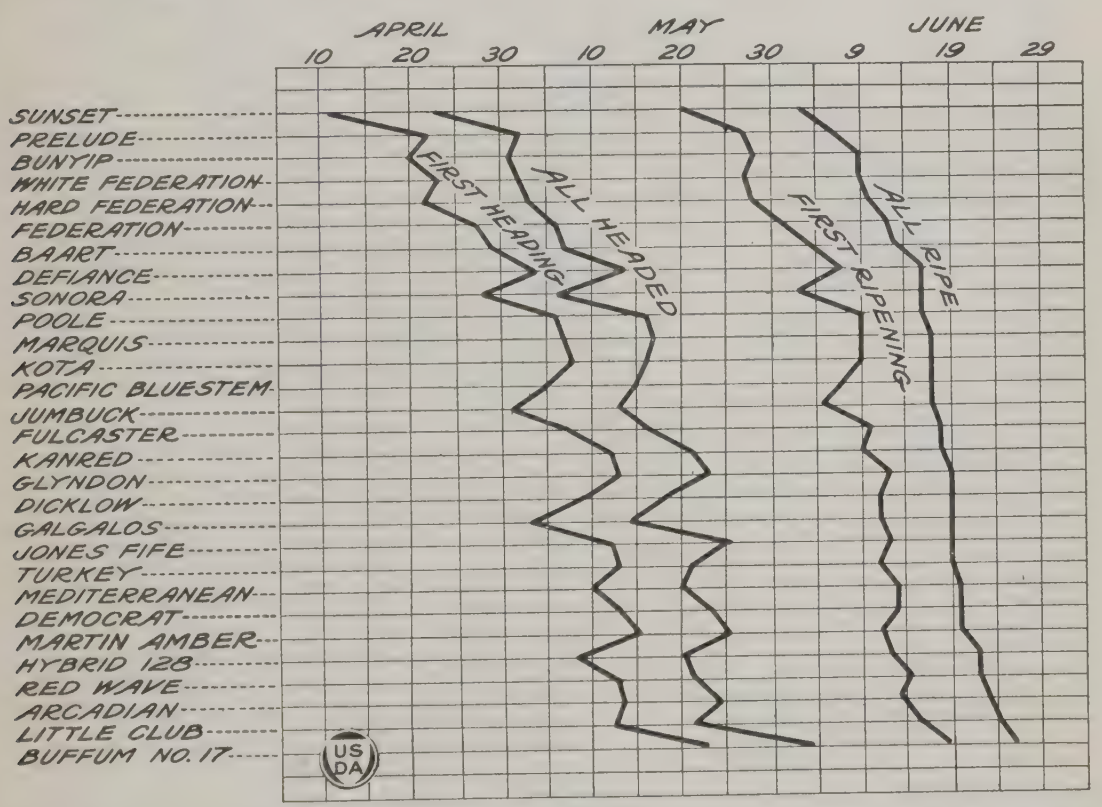

FIG. 1.—Curves showing dates of first heading, all headed, first ripening, and all ripe, for 29 representative varieties of wheat grown in the wheat classification nursery at the University Farm, Davis, Calif., in 1922

All varieties in the classification nursery are pure lines, and the date of first heading has been fixed as the time when the tips of the first 3 or 4 heads appear in the pure-line row. The date recorded for the stage of development termed "all-headed" is the time when all heads not influenced by border position were fully exerted. The plants favored by position were those included within the last six inches at each end of the row. Frequently the plants in this location would develop nearly as rapidly as those in the remainder of the row, while at other times their growth may be definitely retarded.

The curves show a comparatively wider divergence of the dates of first heading and all headed than of the dates when the first heads ripened and when all were ripe. The difference, for example, in the date of first heading of Marquis and of Sunset was 25 days; in date of first ripening it was 17 days; and in date when all were headed it was 15 days. Figure 1 shows that most of the varieties present a relatively similar divergence. Little Club, when compared with Arcadian, is an exception in that it heads earlier but ripens later than Arcadian. Farrer (*6*) also made the observation that the variety of wheat that heads first is by no means the first to ripen. It may be noted that this behavior is exceptional. Club varieties are abnormal in their behavior since they require a longer fruiting period than the common wheats.

Figure 2 gives heading and ripening data for the same 29 varieties grown at Chico, Calif., in 1920. In this season the difference in time of first heading of Sunset and Marquis was 28 days; in

date of first ripening, 16 days; and in date when all were ripe, 13 days. In general, the Chico data agree very closely with the Davis data, except that the high temperatures reduced the length of the ripening period.

EXPERIMENTAL PROCEDURE AND DATA

The index of earliness used in the studies reported in this paper was the date of emergence of the tip of the first spike. This is believed to be the most dependable index for studying earliness in cereals under California conditions.

PARENT VARIETIES

The cross used in this study was between a pure line of Marquis and a pure line of Sunset, the latter being the earliest of the Australian varieties of wheat introduced thus far. In a collection of over 1,400 foreign and domestic wheats, including a considerable number of early wheats from India, grown in 1920 at the United States Plant Introduction Station, Chico, Calif., Sunset was the first variety to head. Marquis is considered a moderately early variety throughout the northern spring-wheat belt of the United States and Canada, but when

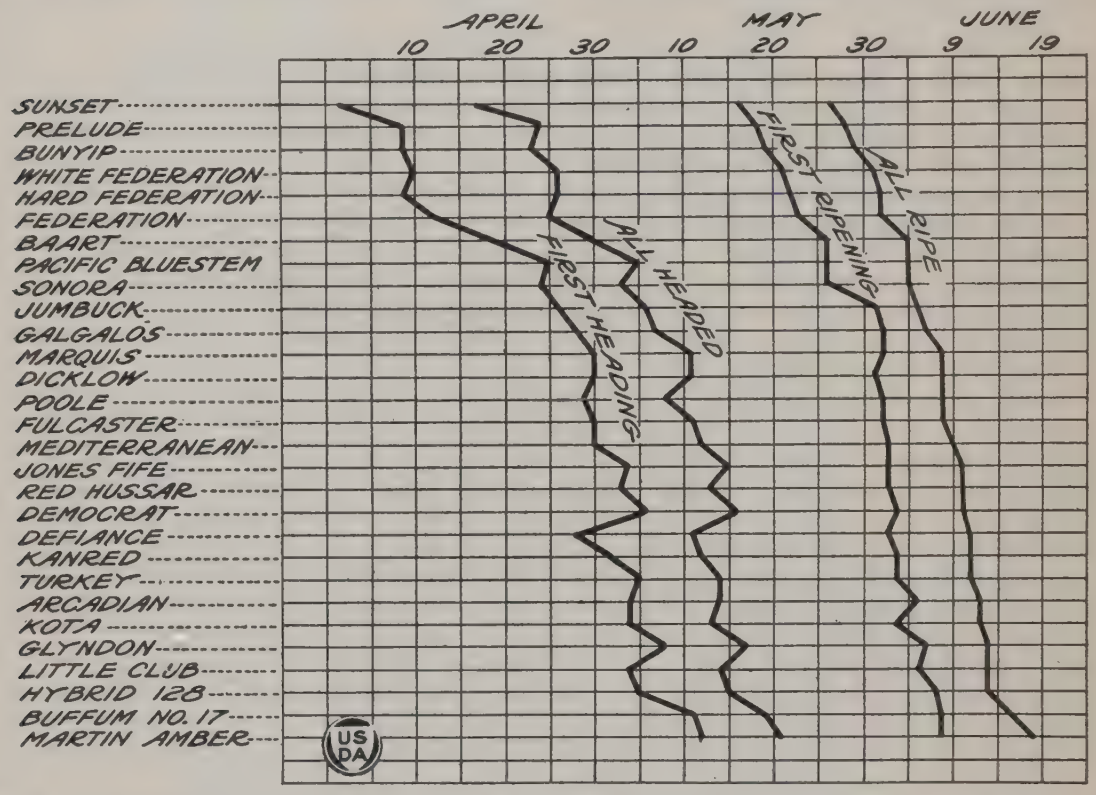

FIG. 2.—Curves showing dates of first heading, all headed, first ripening, and all ripe, for 29 representative varieties of wheat grown in the wheat classification nursery at the Plant Introduction Station, Chico, Calif., in 1920

The date of first ripening was taken as the time when the tip spikelets of the 3 or 4 earliest heads in the row began to take on the natural color of maturity and their kernels began to show a definite stiffening or hardening. It may be added that the color of maturity of the chaff varies. In white-glumed varieties it is nearly white to white or yellowish. In brown-glumed varieties it is yellowish brown to brown. The date on which the plants were recorded as all ripe normally is the time when all heads in the row were fully ripe, except those subject to border effect, which were not considered. At this stage the kernels were hard and the color of the glumes had changed to normal at maturity.

grown in California it is quite late in comparison with Sunset.

P_1 MATERIAL AND F_1 PROGENY

The cross, Marquis × Sunset, and its reciprocal, was made in 1920 at Chico, Calif. In making the cross two spikes from each variety were used, and the pollen was obtained at random from each of the parent rows. Nine apparently crossed kernels were obtained from the cross Marquis × Sunset and 21 kernels from the reciprocal Sunset × Marquis.

The F_1 generation was grown at Chico. The date of first ripening of this population as a group was noted to be slightly earlier than that of the Marquis parent.

TABLE I.—*Frequency table showing distribution of the parents and the F_2 progeny of the Marquis×Sunset and Sunset×Marquis crosses by dates of first heading in head rows at the University Farm, Davis, Calif., in 1922*

Marquis×Sunset and Reciprocal	Dates of first heading																																		Totals
	April																							May												
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	
Sunset Parent						1		4	2	1	2	1	7	4	1	4	1	1	1											3	8	6	5	6	
Marquis Parent																																			
Marquis×Sunset:																																			
20166A1														4	6	2	5	1	3	2	1		1			4		1							30
A2												1		3	2	4	1	3	1	4	3	2	4		1		2	3	1	3		1				39
A3	1			1								1	1		4	3	3	2		3	4		1			1	3	2								30
A4												3	5	1	10	4	3	5				1	2	1		1	3	1	1	1						42
B1												1	3	7	5	2	1		1	2	2	1	1	2	4			2		1						35
Sunset×Marquis:																																			
20167A1											2	3	3	1	5	4	1	1	1				1	3	1	1	2	5				1			35
A2												3	2	2	4	5	6	4		2	1		1		1	2		3								36
A3										1	2	2	2	8	4	6	1	1		2			1			2		1								33
A4								1	1	1		2		3	5	2	3	2		1	1		1		2		1	2	1							29
A5													3	4	3	2	4	2	2	2	2			2	1	2		1								30
A6									1		2	2	1	3	4	4	1	4	1	1				2	1	2	2		1							32
A7											1		2	4	3	5	2	1	3	1					2					1						25
A8									1			3	5	1	6	5	3	2					1	1	1	1			1							31
A9								1		1			4	4	6	5	4	2	2	2	1		1	1	1	3	3									41
A10									1	1	1	3		2	3	4	6	3	2	1	2			1					1		1					32
A11												2	3	5	8	4	5	2	1		2	1		3	3	3	2	1								45
A12								1	1		2	1	7	4	4	5	4	2							3	1	1	2		1						39
A13											1		3	3	5	7	6	3	4	2	1	1	1		2	3										42
A14														1	3	5	3	2		1			1	3			1	1								27
B1								1		1	1	3		1	3	5	3	2		1							2	2								41
B2												1	4	8	2	3	5	2	4	4	2				1	1										28
B4									1		2		4	2	4	2	2	3	2		1			1	1	1			2							35
B5										1	2		2	2	1	3	3	1	2		5		1	6	1	1	1		1	1	1					35
												4	2		5	2	5	4		1		2	1	1	2	4		2								35
Totals	1			1			1	3	7	6	16	35	56	72	102	88	77	52	29	31	28	8	19	27	25	34	23	28	11	7	3	2			792

F_2 PROGENY

In the fall of 1922 the kernels from one average head of each of the F_1 plants were sown at the University Farm, Davis, Calif. The kernels were spaced 2 inches apart in the row. The date of emergence of the first head on each plant was noted. Seven hundred and ninety two hybrid plants, representing 23 families, were tagged for first heading. A frequency table of these data, showing the variation in time of heading, is given in Table I, and a frequency polygon is shown in Figure 3. The frequency curve shows a so-called "heaping up" of individuals into a large group of fairly early plants and a smaller group of rather late plants, with a distribution covering almost the entire range of the parents. A few plants were earlier than the earliest plants of the early parent, while only one was as late as the latest plants of the late parent. Considering the family progeny in each row, in practically every case there was a definite segregation into an early group and a late group, with a distribution of each closely approximating a normal frequency curve. A combination of all families represented shows that there is some overlapping of the two distributions. If an arbitrary division between early and late groups is made at April 28, which is the minimum frequency class in this part of the curve, and half of the 8 individuals therein are assigned to each group, there will be 609 plants in the early group and 183 plants in the late group, which is a ratio of 3.07 : 0.93. This indicates a one-factor difference for earliness in the two varieties. The deviation from the 3:1 ratio probably is not significant, for it is less than twice the probable error, ± 0.04.

Figure 3 gives the curve of the maximum daily temperature during the heading period of the cross. The maximum temperature gives a fairly good measure of the prevailing daily temperatures at this time. These data are included to show that low temperatures were not the cause of the low frequency of first heading on April 28, and the consequent bimodal form of the curve.

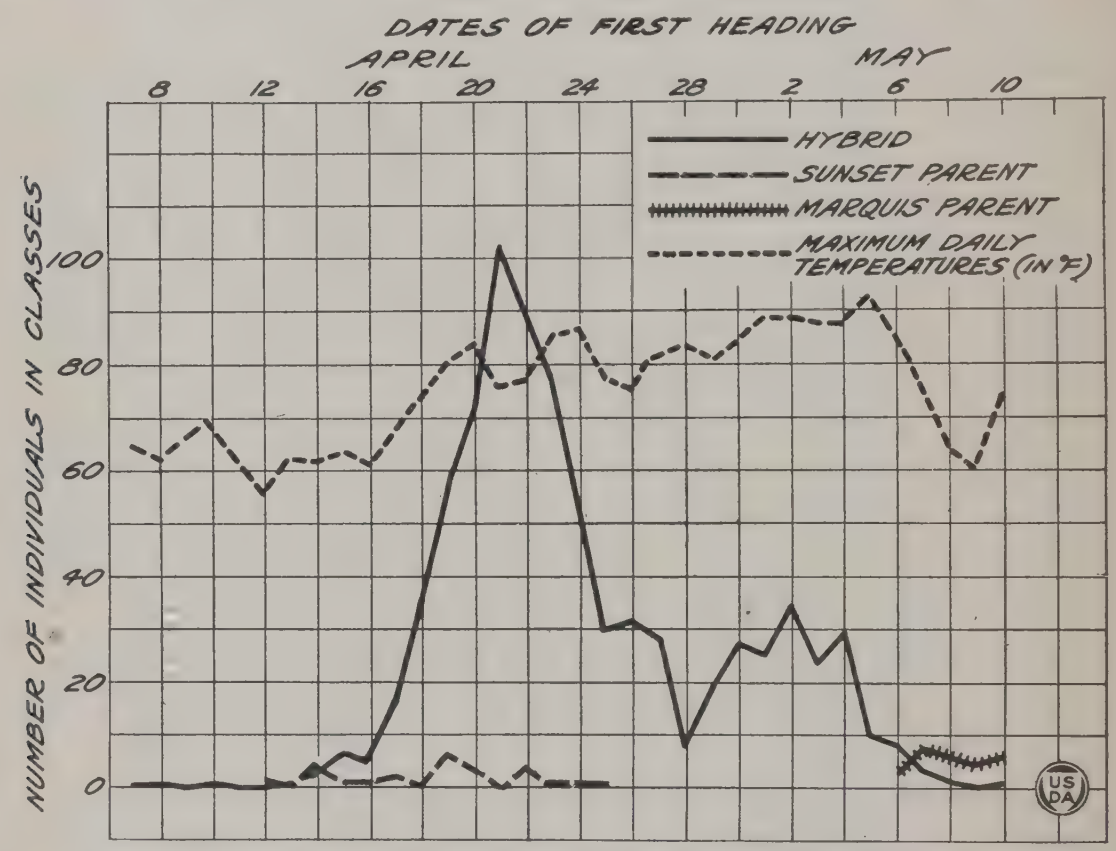

FIG. 3.—Curves showing the frequency distribution of the F_2 hybrid population, the Sunset parent, and the Marquis parent, by dates of heading, and the maximum daily temperature for the heading period in degrees Fahrenheit, at the University Farm, Davis, Calif., in 1923

F_3 PROGENY

The sowings for the F_3 progeny were made in the same way as those for the F_2. One head from each of the F_2 plants was harvested and the kernels were sown 2 inches apart in rows 5 feet long and 16 inches apart. The index for earliness, however, was obtained in a different manner. The date of first heading for each individual plant in each row was not noted, but the appearance of the first head

in each row was recorded, as well as the date when the last head made its appearance. This gave the date of first heading of progeny from each F_2 plant, as well as the interval of time required from first heading until all plants were fully headed.

As a wheat plant is considered a unit in heredity, any spike from that plant will exhibit in its homozygous progeny the characters of its parent. A homozygous head row from a recessive late plant will begin heading late; a homozygous early head row will begin heading early; and a heterozygous head row will begin heading early, as it contains both early and late plants. In the F_3 generation, therefore, a similar distribution for first heading should be obtained as in the F_2. Table II gives the frequency distribution of date of first heading of the F_3 head rows.[4] There is a sharp segregation into an early group and a late group almost identical with that in the F_2 generation.

If the division is made between the April 2 and April 3 dates of first heading in the F_3, the totals in the early and late classes are 607 and 184, respectively, or practically the same as were placed in the early and late classes in the F_2. These totals are not made up entirely of the same individuals as made up the early and late classes in the F_2, however, as about 4 per cent of the segregates have shifted back or forth with respect to date of first heading.

USE OF HEADING DATA AS A METHOD OF ANALYSIS OF F_3 ROWS

A further analysis of the F_3 population was made to determine, if possible, the segregation for rows homozygous and those heterozygous for duration of heading.

In taking agronomic notes on pure-line varieties it has been observed that within a group, such as common wheat, club wheat, etc., the period from first heading to fully headed is fairly uniform and constant (figs. 1 and 2). On account of a number of factors, such as lower temperatures, shorter period of daylight, etc., varieties heading early in the season require a somewhat longer period to progress from first heading to fully headed than do those coming later, but those heading at about the same time require approximately an equal time for this period of development. It should be possible in segregating hybrid material, therefore, to separate the early and late homozygotes from the heterozygotes by the length of time required to come from first heading to the fully headed stage. The homozygotes would require a comparatively short time to complete the heading process, while the heterozygotes would require a longer time, in some cases extending over the entire season, depending upon the degree of heterozygosity.

The season of 1923 at Davis was marked by high temperatures in February and March, so that the progeny rows from the early F_2 segregates began heading in late March. A heavy rain followed by cooler weather occurred from March 30 to April 2. This retarded the heading of the late segregates and slowed down the progress of those already heading. However, most of the progeny rows from the early segregates began heading before the storm occurred, so that earliness of heading probably was not affected much in this group, and the late segregates probably were affected about equally.

USE OF TIME-TEMPERATURE EFFICIENCY UNIT AS A METHOD OF ANALYSIS

To get the most accurate measure of the time required for heading in the various rows, it was decided that temperature also must be considered, as it materially affects the rate of plant growth. High temperatures with rapid development occurred both early and late during the heading period, while in the intermediate period the rate of heading was slower. The time required for heading therefore was converted into time-temperature efficiency units. A time-temperature efficiency unit was taken arbitrarily to mean 1° F. for the duration of one hour above a certain basic temperature, below which the wheat plant probably makes no appreciable growth.

The minimum temperature of growth for the seedling wheat plant was found by Sachs (*14, p. 365*) to be 41° F., the optimum temperature, 83.7° F., and the maximum temperature, 108°. Other investigators have found that the growth of many plants ceases with falling temperatures at 40° to 43°. In growth-temperature studies with plants, 40° has been the basic temperature commonly used. It will be considered in this study also as the temperature below which little or no growth occurs in wheat. The optimum temperature was taken as 83.7°, as determined by Sachs, although it is probable that plants approaching maturity are not so sensitive to higher temperatures as seedling plants.

[4] There were 792 head rows in the F_2 (Table I), but the seed from one failed to germinate, hence 791 in F^3.

TABLE II.—*Frequency distribution of F_3 progeny in head rows by dates of first heading of early and late segregates from the F_2.* (*Early segregates above heavy line; late segregates below*)

Date of first heading in F_2 (1922)	Dates of first heading in F_3																																	Totals
	March							April																											
	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Apr. 7																										1										1
8																																				
9																																				
10						1																														1
11																																				
12																																				
13			1																																	1
14		1	2																																	3
15		2	3	2																																7
16			3	2			1																													6
17		1	4	7	2	1																								1						16
18		2	7	17	9																															35
19		1	9	30	6	7	2		1																											56
20		3	7	32	13	10	5	1																						1						72
21		2	11	46	12	22	8	1																												102
22			7	42	16	12	7		2				1													1										88
23			7	24	14	15	11	2	2				1														1									77
24		1	3	15	9	11	8	2	2																1										52
25				6	2	9	4	2	4				1										1													29
26		1		8	7	4	7	1				1											1													30
27				6	2	12	3		1	1								1				1								1						28
28				1	2	1	1						1					1		1																8
29				3		1	1	1			1		2			1		1		2		4	2													19
30							1		1					1					1	5	7	3	5		1	1	1									27
May 1								1										1		1	4	2	11	1	2	2									25
2								1													8	3	8	2	6	6									34
3				1																2		3	7	1	2	3	3				1				23
4	1			1		1												2		1	1	2	5	2	2	6	3		1						28
5																					1	1	3		1	2	1		1	1					11
6																						1			1	1	2				2				7
7																					1			1						1					3
8																							1							1					2
Totals	1	14	64	243	94	107	59	12	13	1	1	1	6	1		1		6	1	12	22	20	44	7	16	23	11		3	5	3					791

In calculating the time-temperature efficiency units it is important to consider the effectiveness of varying degrees of temperature in promoting growth. As plants approach the optimum temperature the growth rate is approximately doubled for each rise of 18° in temperature above the basic temperature in conformity with van't Hoff's law of chemical reaction velocity, as set forth by Livingston and Livingston (*13*). The daily number of time-temperature efficiency units was determined from thermograph records during the entire heading period of 1923. From these data the total number of such units required to bring each row from date of first heading to date of last heading were calculated.

TABLE III.—*Time-temperature coefficients (by exponential system) adapted to hourly record on thermograph at Davis, Calif. (Doubled growth rate assumed for each 18° F. (10° C.), and using only temperatures above 40° F.)*

Degrees above 40° F.	Time-temperature units	Degrees above 40° F.	Time-temperature units
1	1.00	28	2.82
2	1.04	29	2.93
3	1.08	30	3.05
4	1.12	31	3.16
5	1.17	32	3.29
6	1.21	33	3.42
7	1.26	34	3.55
8	1.31	35	3.68
9	1.36	36	3.84
10	1.41	37	4.00
11	1.47	38	4.16
12	1.53	39	4.32
13	1.59	40	4.49
14	1.65	41	4.67
15	1.71	42	4.85
16	1.78	43	5.03
17	1.85	44	4.85
18	1.92	45	4.67
19	2.00	46	4.49
20	2.08	47	4.32
21	2.16	48	4.16
22	2,25	49	4.00
23	2,33	50	3.84
24	2.42	51	3.68
25	2.52	52	3.55
26	2.62	53	3 42
27	2.72		

According to the method of calculation used, the value of a unit at 41° for one hour is 1, at 59° it is 2 for each hour, at 77° it is 4 for each hour, and so on to the optimum temperature. The added increments of efficiency, calculated for each degree, are shown in Table III. At temperatures above the optimum the growth rate was assumed to decrease at the same rate at which it previously had increased.

Table IV gives the daily time-temperature efficiency units for the entire heading period from March 26 to May 10.

Table V is presented for purposes of comparison to show the number of time-temperature units, as arbitrarily defined previously, which are required to bring head rows of parent varieties from first heading to complete heading. Sunset apparently requires from 850 to 1,050 such units, while Marquis, coming later in the season at higher temperatures, requires a smaller number. It will be observed in Table VII that the class of greatest frequency of the so-called homozygous rows is found at 901 to 950 time-temperature units in both the early and the late groups.

TABLE IV.—*Daily time-temperature efficiency units from March 26 to May 10, 1923, at University Farm, Davis, Calif.*

Date	Daily time-temperature units	Date	Daily time-temperature units
Mar. 25	62.5	Apr. 19	51.5
26	61.5	20	46.2
27	66.0	21	37.1
28	62.4	22	45.1
29	52.9	23	47.1
30	51.8	24	58.5
31	44.5	25	70.8
Apr. 1	37.3	26	50.0
2	35.6	27	42.0
3	35.0	28	39.2
4	35.5	29	41.7
5	42.3	30	50.1
6	38.5	May 1	49.4
7	39.3	2	48.9
8	42.9	3	65.5
9	38.9	4	53.9
10	49.8	5	58.5
11	46.0	6	64.1
12	53.2	7	63.3
13	60.3	8	70.4
14	58.6	9	74.1
15	64.9	10	60.8
16	39.0	11	66.7
17	34.8	12	72.6
18	44.8		

TABLE V.—*Total time-temperature efficiency units required to bring the recorded numbers of individual head rows of parent varieties from first heading to complete heading*

Parent varieties	Numbers of efficiency units										
	501–550	551–600	601–650	651–700	701–750	751–800	801–850	851–900	901–950	951–1,000	1,001–1,050
Sunset								5	7	6	1
Marquis	1	6	6	6	2		4				

TABLE VI.—*Frequency distribution of F_3 progeny based on time-temperature efficiency units required to bring F_3 rows of early and late segregates from the F_2 from stage of first heading to stage of complete heading*

Dates of first heading in F_2 (1922)	Time-temperature efficiency units																															Totals
	501–550	551–600	601–650	651–700	701–750	751–800	801–850	851–900	901–950	951–1,000	1,001–1,050	1,051–1,100	1,101–1,150	1,151–1,200	1,201–1,250	1,251–1,300	1,301–1,350	1,351–1,400	1,401–1,450	1,451–1,500	1,501–1,550	1,551–1,600	1,601–1,650	1,651–1,700	1,701–1,750	1,751–1,800	1,801–1,850	1,851–1,900	1,901–1,950	1,951–2,000	2,001–2,050	
Apr. 7									1																							1
8																																
9																																
10																						1										1
11																																
12																																
13							1																									1
14								2		1																						3
15						1	1	1	1	1	1	1																				7
16					1			1	1	1	1						1															6
17								1	4	6	2	2											1									16
18		1						2	10	6	5	3	2					3		1		1				1						35
19					1		2	6	8	9	5	5	2	3	1	2		1	1	5	1	2	1	1								56
20					2		1	9	10	6	4	2	6	3	3	3		3	4	5	3	7				1						72
21							4	2	9	7	7	7	7	1	3	4	7	4	8	19	6	2	2	2							1	102
22						1		3	9	7	6	3	4	2	4	3	8	6	7	15	3	3		1	1			1	1			88
23							1	1	3	4	5	2	2	3	3	4	8	4	6	10	8	5	2	2	1			2	1			77
24					1	1	1	2	4	1	3	1	2		2		3	4	5	8	4	6		1			3					52
25									2	2	2	3	2	1	1	1	2	2		3	2	2	2						1	1		29
26									4	2				1	1	1	4	2	4	3	2	2	2	1			1					30
27								1	4	1	3	1	1	1	2	1	1	2		4	1	3			1			1				28
28							1			1				2				1		2									1			8
29					1	1	1	2	3	1		1		1	1	1		1	3	1	1											19
30			1	1	1	5	2	4	5	2	1			1		1		1	1				1									27
May 1				2	1	5		2	3	1	1	2	4	3				1														25
2				2		2	5	4	7	2	2	4	3	2					1													34
3				1			2	1	2	4	5	3	3		1							1										23
4				1	2	2	3	1	4	4	4	2	2	1		1							1									28
5					1	3		1			1	1	1	1	1			1														11
6						1	1		1		2	1	1																			7
7							1	1			1																					3
8								1	1																							2
9																																
10																																
Totals		1	1	7	11	22	7	48	96	69	61	44	42	26	23	22	34	36	40	76	31	35	12	8	3	2	4	4	3	2	1	791

TABLE VII.—*Frequency distribution of F_3 head rows based on time-temperature efficiency units required to bring* ***each head row*** *from the date of first heading to complete heading*

Dates of first heading	Groups of time-temperature efficiency units in degrees F.																															Totals
	501–550	551–600	601–650	651–700	701–750	751–800	801–850	851–900	901–950	951–1,000	1,001–1,050	1,051–1,100	1,101–1,150	1,151–1,200	1,201–1,250	1,251–1,300	1,301–1,350	1,351–1,400	1,401–1,450	1,451–1,500	1,501–1,550	1,551–1,600	1,601–1,650	1,651–1,700	1,701–1,750	1,751–1,800	1,801–1,850	1,851–1,900	1,901–1,950	1,951–2,000	2,001–2,050	
1923																																
Mar. 25						1		3		2		2					1	1	1			3										14
26					5		3	9		11	5	1	5	3	1	1	2	3	5	2	3	4	1				1		1			66
27						1	2	8	38	22	32	21	10	5	12	8		2	13	43	9	12	2	4		2		3				249
28		1						4	14	7	2	2	2	6	5			11	12	5	8	4	3	2	2		1			1		92
29							2	4	11	6	1	3	5	1		2	21	9	2	24	4	5	3	3	1		2		2		1	112
30						1	3	2	3	1	3	1	2		1	6	8	4	4	3	3	6		1			2	1	1			56
31										2						2	2	1	2		4											13
Apr. 1									1	3	2			1	1	1	1					1	2									13
2															1																	1
3																				1												1
4																							1									1
5												1		1			1	3														6
6																																
7																		1														1
8						1						1																				2
9																																
10									1	2		2	1			1																6
11																1																1
12							4	4	1	3						1																14
13						3	4	3	6	1	2	2		2	1	2																26
14					1	3	1	4	2	1	3		2	1	3																	21
15			2	5	1	7		2	7	5	6		8	4																		47
16						3		2	3				3																			11
17					2	1	5				4	4																				16
18				1	1	2		3	6	6		7																				26
19								1	4		4	2																				11
20																																
21						1	1		1																							3
22					1			3	1																							5
23						1	2																									3
24				1																												1
25																																
26																																
27					1																											1
28																																
Totals		1	2	7	12	25	27	52	99	72	64	49	38	25	25	24	36	35	39	78	31	35	12	10	3	2	6	4	4	1	1	819

Table VI gives the frequency distribution of F_3 head rows by date of first heading of the F_2 parental segregates and by time-temperature efficiency units required to bring F_3 head rows from date of first heading to complete heading. Figure 4 gives a distribution curve of these data. If the one-factor hypothesis for earliness is correct, there should be an equal number of homozygous and heterozygous rows in the F_3 progeny, according to the symbols $AA+2Aa+aa$, where A equals the factor for earliness and a that for lateness. The total number of individuals observed in this group was 791. When an arbitrary division at the minimum frequency class of 1,251–1,300 time-temperature efficiency units is made and this class included with the heterozygotes, 481 rows, or 60.70 per cent, were homozygous and 39.30 per cent heterozygous, or a ratio of 1.21 : 0.79, which indicates the expected 1 : 1 ratio. By shifting the division slightly to the left this ratio can be obtained almost exactly.

When the division is made at the 1,251–1,300 class, 21 progeny rows of the late segregates in the F_2, or about 11 per cent, are classed as heterozygous. In addition, certain F_2 plants which began to head about midseason gave progeny which were apparently fully homozygous in the F_3. Also 293, or 48.2 per cent, from a total of 609 plants classed as early in the F_2, fall into the heterozygous class. This is nearly a 1 : 1 ratio, whereas that expected is 2 : 1. The deviation from the expected ratio may indicate that the division between homozygous and heterozygous head rows has been placed too high. Furthermore, it may indicate some shortcoming in the method of calculating time-temperature efficiency units. Another possible cause is the difficulty experienced in determining accurately the end point for heading when taking notes.

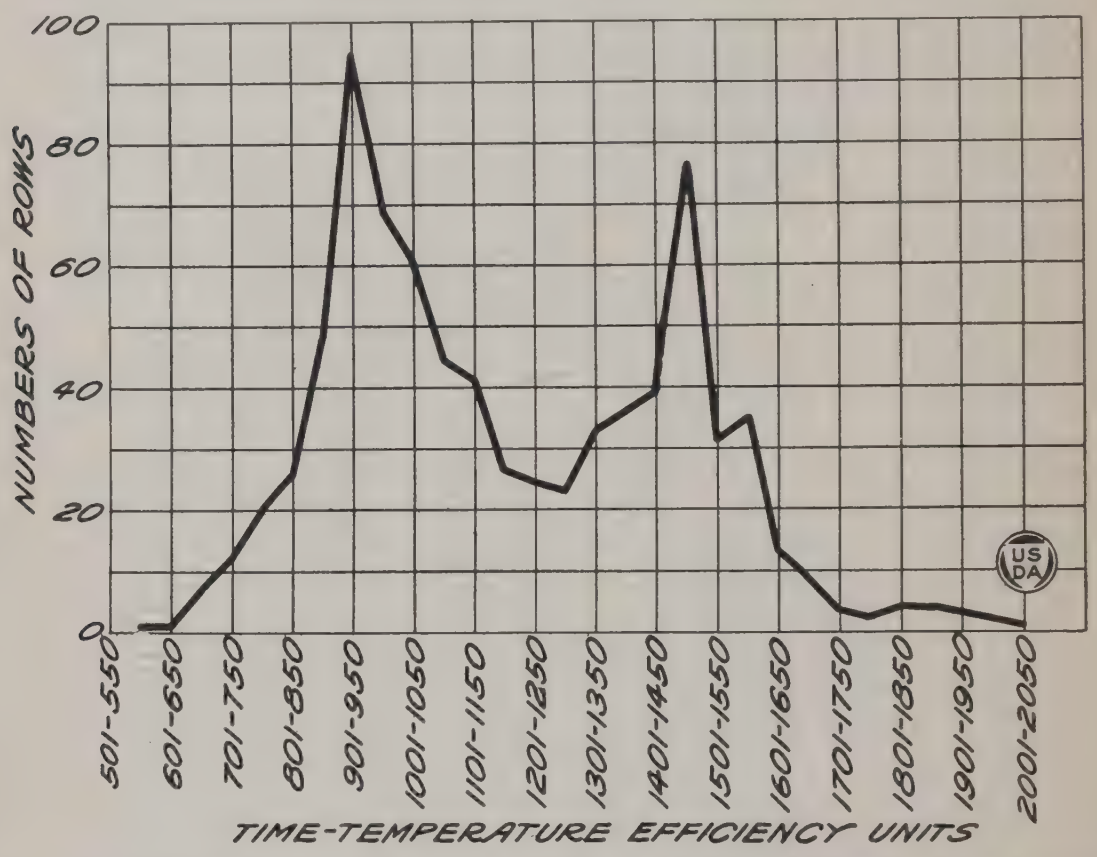

FIG. 4.—Curve showing frequency distribution of F_3 head rows by date of first heading of the F_2 parental segregates and by time-temperature efficiency units required to bring F_3 rows of early and late segregates from the F_2 progeny from first heading to complete heading

Table VII gives the frequency distribution of the F_3 head rows by date of first heading of these rows and by total time-temperature efficiency units required to bring each head row from the date of first heading to the date of complete heading. This table shows a definite segregation into a homozygous late group. Of the total of 819 individuals (including 28 hybrid plants of this cross not tagged for first heading in F_2), 193, or 23.41 per cent, fall into this group. For the whole population this makes a ratio of 3.08 : 0.92, which agrees closely to a 3 : 1 ratio. The total number of individuals in the early group was 627.

TABLE VIII.—*Frequency distribution of the F_3 head-row population with reference to the number of days required for each row to pass from first heading to last heading*

	Number of days																													Total
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
Number of rows	1	8	10	22	30	30	57	82	69	77	37	22	25	14	21	26	34	47	34	67	38	30	8	10	5	6	4	3	1	1	819

Those classed as early began heading before April 10. Of these, 310 were homozygous and 317 were heterozygous. The minimum frequency class (ordinate 1,251–1,300) was included with heterozygotes. This makes a ratio of 1.00 early to 1.02 late, while the expected ratio is 1 : 2. The reasons assigned above for the discrepancies observed may also apply here.

per row. While the curves from the two sets of data are similar in general outline, a more definite segregation is shown by the latter. The class of greatest frequency in the homozygous group, which developed in the period of low time-temperature units, contains 95 compared with 82 in the same group when the distribution is shown by the days required to develop from first heading to complete heading.

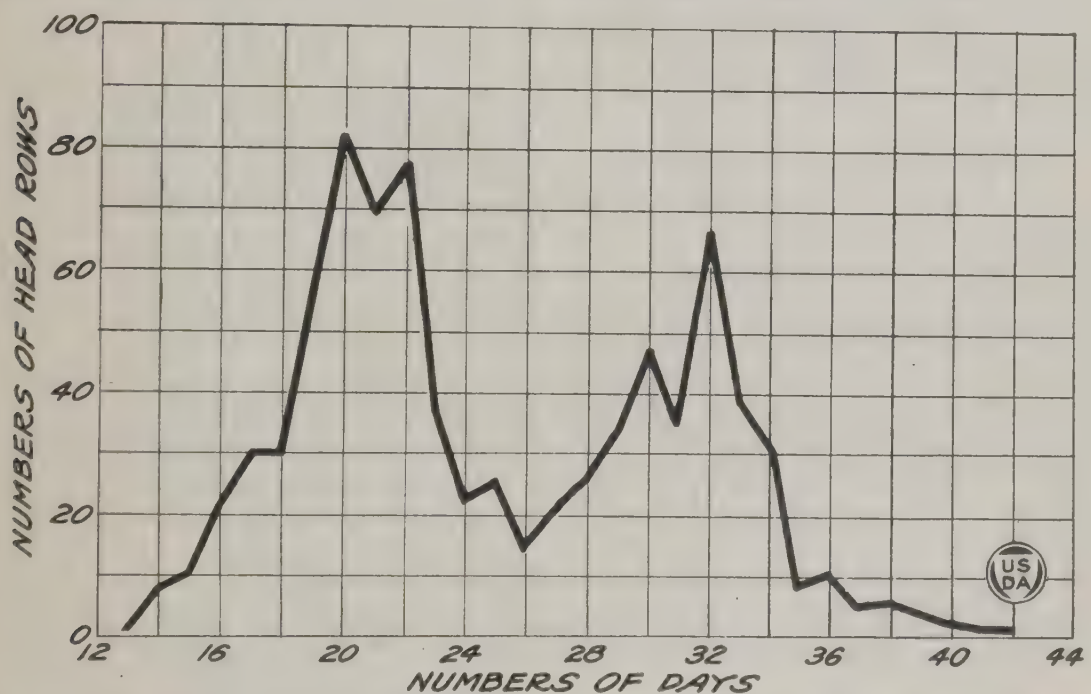

FIG. 5.—Curve showing frequency distribution of the F_3 head-row population based on the number of days required for each row from first heading to last heading

In Table VIII and Figure 5 are shown the distribution of the F_3 head-row population based on the number of days required by each row to develop from the appearance of the first head to the stage when all heads were out. There is shown a large group requiring a comparatively short period and a smaller group requiring a comparatively long period for this development. The division evidently is at 26 days. When half of the individuals of this class were assigned to each group, 58.25 per cent of the rows appeared homozygous and 41.75 per cent appeared heterozygous. This is a ratio of 1.19 to 0.83, which is approximately the same as the segregation on the basis of the total time-temperature units required

Inspection of the data shows that rows requiring the same number of time-temperature units in a number of cases show quite a wide divergence in the total number of days required. This indicates that there was a distinct slowing down of plant growth due to unfavorable temperature conditions, which should be taken into account in a study of this kind. Although a consideration of the time-temperature relation apparently permits a somewhat more accurate analysis of the genetic factors involved in the inheritance of earliness than time alone, it is evident that more information is needed concerning the response of the wheat plant to temperature and other factors which influence growth.

DISCUSSION

EARLINESS IN THE F_1 GENERATION

According to the investigations reviewed earlier in this paper, in two cases (*6*, *3*) the time of maturity in wheat crosses was intermediate in the F_1, while in two others (*2*, *15*) it was inclined to the late parent. In experiments with oats (*4*), corn (*5*), and cotton (*12*), the time of maturity was found to be intermediate; in rice (*10*) it was intermediate but inclined to the early parent; in experiments with peas it was found to be intermediate in one (*1*), while in the other (*10*) it was inclined toward the late parent. The exact behavior in the F_1 was not recorded in the Marquis × Sunset cross reported in this paper. It is very probable that time of maturity is intermediate and possibly inclined toward the early parent, as behavior in the F_2 and F_3 shows earliness to be dominant.

EARLINESS IN THE F_2 GENERATION

The curve of the data for the F_2, as shown by Figure 3, indicates strongly the presence of one main allelomorphic pair of factors for earliness in this cross, as there is a distinct segregation of the population into a large early group and a small late group in a ratio of nearly 3:1 (3.07:0.93). The modes of the two groups diverge from those of the parent varieties toward the intermediate position, which would indicate the presence of modifying factors also. The class of greatest frequency of the early group is only two days later than that of the early parent, whereas in the late group it is five days earlier than that of the late parent.

EARLINESS IN THE F_3 GENERATION

If the inheritance of earliness in this cross can be explained by a single pair of factors, the recessive late group should breed true in the F_3. Nearly 93 per cent of the recessive late individuals from the F_2 were late in the F_3 and, considering the total population, the ratio of early to late was 3.09 to 0.91, which indicates that a one-factor hypothesis for this cross is correct. Further evidence in favor of this view is furnished by the fact that over one-half of the F_3 rows were homozygous for earliness, as shown by the time-temperature unit method of analysis according to segregation in the F_2, $AA+2Aa+aa$.

This experiment is subject to a certain amount of error from various sources. Late or premature heading may be caused by variation in the amount of available soil nutrients, poor drainage, disease, mechanical injury, etc., but with considerable numbers these factors should have little or no effect on results. The index for the end of the heading period is less definite and more difficult to obtain where material is not sacrificed, as was the case in this experiment. Some difficulty was encountered in connection with second-growth culms or tillers which appeared in the late segregates after the spring rains, but these were quite successfully disposed of by ignoring such culms below the knee in height. Close examination was also necessary on account of the large number of culms in each row, yet it is believed that fairly accurate data were procured in this study.

SUMMARY

The cross Sunset × Marquis and its reciprocal were used in this study. The Sunset parent, an Australian variety, is one of the earliest wheats known. The Marquis parent, the well-known hard spring wheat grown in Canada and the northern United States, is a midseason to late variety in California.

The cross was made at Chico, Calif., in 1920. The F_1 was grown at Chico in 1921, and the F_2 and F_3 generations at Davis, Calif., in 1922 and 1923.

The inheritance of time of maturity in wheat and other plants has been studied by a number of investigators. The F_1 has been found to be intermediate or inclined to one or the other parent. In the F_2 there has been a tendency for the intermediate condition to be maintained with a range of variation almost covering the extremes of the parents and both with and without the grouping into classes showing definite segregation.

It is believed that the best index in a study of earliness and lateness in wheat is the date of appearance of the tip of the first spike on the plant. Slight differences are more magnified at this time than at the period of ripening.

No data were secured on the F_1 generation. Other investigators have shown that time of maturity in wheat is either intermediate or inclined to the late parent in this generation.

In the F_2 there was a distinct heaping up of the population into a large early and a small late group in the proportion of 3.07 to 0.93, indicating one allelomorphic pair of factors, with possibly a number of minor modifying factors.

The recessive late group of the F_2 remained late in the F_3, tending to confirm the presence of only one pair of factors for earliness as contrasted with lateness. Further evidence of this view was furnished by the fact that more than one-half of the F_3 population apparently was homozygous for earliness.

These studies indicate that earliness or lateness in wheat is a definitely heritable character, and reports by various investigators show it to vary in complexity in different combinations. Within the common wheat group the character for earliness in winter (habit) × spring (habit) varieties apparently is more complex than in crosses between two spring (habit) varieties.

It is possible to determine progeny rows homozygous for earliness in F_3 and subsequent generations of hybrids by the time required for them to pass through the heading stage. This conclusion is based on the assumption that pure-line rows pass through the heading stage in a time approximately equal to that of pure-line parent varieties.

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THE VITALITY OF BURIED SEEDS [1]

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The distribution and perpetuation of plants depends largely upon seeds. As far back as anything is known of the human race, man has used seeds for plant propagation. Comparatively few species of plants contribute directly to the needs of man, and he has chiefly interested himself in these few. The great remaining flora depends for its reproduction and distribution upon natural conditions. Many plants have effective devices for the scattering of their seed, and often the seeds themselves are safeguarded by a dormant or resting period which prevents them from germinating until the occurrence of favorable seasonal conditions. How long seeds are capable of remaining viable has always been an interesting question, and extravagant statements have frequently been made regarding the germination of seeds of great age. The general impression has prevailed that seeds of various plants are able to retain their vitality for long periods, although buried in the soil. There is in the literature frequent mention of plants resulting from bringing to the top former surface soil which for some reason was buried for a period of years.

Such statements and a desire to obtain accurate data prompted Ewart (*4*)[2] to test for germination many herbarium samples of seed of known age. Many of the seeds which grew were over a hundred years old.

The Gardener's Chronicle in 1894 (*1*) cited a case of a charlock-infested field which was put down to grass and permanent pasture. After 23 years a loaded wagon drawn across the field in the spring when the ground was soft brought subsoil to the surface, and in the following summer a ribbon of charlock grew in the wheel tracks. Peter (*7*) obtained soil samples from various depths in several old forests where the soil had not been disturbed for many years. From these samples he was able to grow seedlings and later to identify the resulting plants. Other similar cases indicate that many seeds can remain alive in the soil, but the length of time is largely speculative.

The first attempt, so far as is known to the writer, to obtain specific data on the length of time seeds are able to retain their vitality while buried in the soil was made by the late Dr. W. J. Beal, of Michigan Agricultural College. In 1879 he buried at East Lansing, Mich., 20 inverted open-mouthed bottles, each bottle containing 50 seeds of each of 20 species. One of these bottles has been taken up every 5 years, the last report (*2*) being made after 40 years, at which time 10 of the 20 species produced sprouts.

Munerati (*6*) planted definite numbers of seeds of several species near the surface of the soil in small squares in an open field which had been kept free from weeds for a number of years. He removed the seedlings as they appeared, keeping a monthly record of the percentage of sprouts. After six years some apparently sound seeds were dug up. Kozma (*5*) buried seeds at varying depths (8, 15, 30, and 50 cm.) in sandy and in loamy soil and recorded the sprouts as they appeared during four and a half years. He compares the effect of loamy and sandy soil on vitality and on the ability of the several species to come up through the different soil depths. In 1902 Dr. J. W. T. Duvel, of the Seed Laboratory, U. S. Department of Agriculture, started the buried seeds experiment, the progress of which this paper describes. He followed the general plan of Dr. Beal but used seeds of a larger number of species and subjected them to more natural conditions.

For the experiment Duvel used 112 samples of seed, representing 107 species. A fixed number of each kind of seed was mixed with sterilized soil taken from the pit where the seeds were to be buried. The mixture of seed and soil was placed in common flower pots. These pots, each covered with a porous saucer, were arranged in sets and buried in the soil at Arlington Experi-

[1] Received for publication June 30, 1924—issued January, 1925

[2] Reference is made by number (italic) to "Literature cited," p. 362.

ment Farm, Rosslyn, Va. As a result of this method, the soil within the pots when taken up has resembled, in moisture and compactness, the soil outside the pots.

In all, 32 sets of pots were buried. The trench in which the pots were buried was approximately 9 feet wide at the top and 50 feet long. On one of its sides the soil was removed to a depth of 8 inches; through the middle the soil was removed to a depth of 22 inches; on the other side it was removed to a depth of 42 inches. Thus the trench had three distinct levels or shelves, each about 3 feet wide, of undisturbed soil. The sets of pots were arranged on each of these shelves, 8 sets on the shallow or A level, 12 sets on the middle or B level and 12 sets on the deep or C level. This arrangement made it possible to take up a set from each of the different depths by digging across the trench. After the pots were removed the earth was immediately replaced to prevent any unnecessary disturbance of the remaining sets. Inasmuch as not more than a foot of soil separated the different sets it was impossible to dig up one set of pots without changing somewhat the temperature, aeration and moisture conditions of the soil surrounding the adjoining set.

The seeds upon being dug up have been tested immediately for germination in the greenhouse. The method employed for making the viability tests has been to sift the contents of each pot on a section of a greenhouse flat nearly filled with sterilized soil. Each flat was divided by partitions into four sections. This arrangement permitted the planting of the same kind of seed from each of the three depths, and a check in the same flat.

A description of the experiment and the results obtained at the end of the first year were published by Duvel (*3*), who continued to supervise the experiment through the test of 1912. The viability tests of 1905, 1908, and 1912 were made by the writer, who since then has continued the experiment. The results of all germination tests are given in Table I.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
		POACEAE (GRASS FAMILY)								
16173	31	*Agropyron repens* (L.) Beauv. (couch grass).	200	A	20.5	0	0	0	0	0
			200	B	73	1	0	0	0	0
			200	C	66.5	19	0.5	2	0	0
16174	9	*Avena fatua* L. (wild oats)	100	A	9	0	0	0	0	0
			100	B	8	0	0	0	0	0
			100	C	18	0	0	0	0	0
16175	8	*Avena sativa* L. (oats)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16176	36	*Bromus secalinus* L. (cheat, chess).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16177	37	*Bromus racemosus* L. (upright chess, smooth brome-grass).	200	A	0	0	11.5	0	0	0
			200	B	0	0	42	0	0	0
			200	C	0	0	18.5	0	0	0
16178	66	*Chaetochloa verticillata* (L.) Scribn. (foxtail).	200	A	29	48.5	39.5	30	8	7.0
			200	B	35.5	47.5	37.5	35	3	2.5
			200	C	45	61.5	43	47.5	1	2
16179	33	*Chaetochloa glauca* (L.) Scribn. (yellow foxtail).	200	A	1	2.5	5.5	3.5	0	0
			200	B	1	1.5	1.5	4.5	1.5	0
			200	C	1	2	1.5	1	0	0.5
16180	67	*Chaetochloa viridis* (L.) Scribn. (green foxtail).	200	A	0	55.5	16	38.5	28.5	1.5
			200	B	0	58.5	13	26	5	5.5
			200	C	0	67.5	26.5	79.5	1.5	26
16181	72	*Eleusine indica* (L.) Gaertn. (wire-grass, crab-grass).	200	A	0	3	0.5	0	0	0
			200	B	0	3	1	0	0	0
			200	C	0	2.5	1	0	0	0
16182	15	*Elymus virginicus* L. (Virginia wild rye).	200	A	2	0	0	0	0	0
			200	B	13.5	0	0	0	0	0
			200	C	25.5	0	0	0	0	0
16183	13	*Elymus canadensis* L. (nodding wild rye).	100	A	0	1	0	0	0	0
			100	B	7	1	0	0	0	0
			100	C	22	0	0	0	0	0
16184	14	*Elymus triticoides* Buckl. (wild wheat).	200	A	1.5	0	0	0	0	0
			200	B	3.5	0	0	0	0	0
			200	C	15.5	0	0	0	0	0

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
POACEAE (GRASS FAMILY)—Continued										
16185	35	*Festuca elatior* L. (meadow fescue).	200	A	0.5	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16186	12	*Hordeum sativum* Jessen (barley).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16187	32	*Panicum virgatum* L. (tall, smooth panicum).	200	A	3.5	7	0	0	0	0
			200	B	8.5	14.5	0	0	0	0
			200	C	8	14.5	0	0	0	0
16188	34	*Phalaris arundinacea* L. (reed canary grass).	200	A	45	39.5	29.5	12.5	12	5
			200	B	46.5	61.5	52.5	46.5	6.5	11.5
			200	C	56.5	62.5	38	15.5	0	1
16189	68	*Phleum pratense* L. (timothy)	200	A	0	0	34.5	22.5	3	0.5
			200	B	0	0	56	22	1.5	5
			200	C	0	0	46.5	51	6	12.5
16190	73	*Poa pratensis* L. (Kentucky bluegrass).	200	A	16	42	0	17	17	13
			200	B	22	65.5	21	4.5	27	10.5
			200	C	24.5	80.5	46.5	3.5	0	18.5
16191	11	*Secale cereale* L. (rye)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16192	69	*Sporobolus airoides* Torr. (hair-grass drop-seed).	200	A	0	0	0	0	0	0
			200	B	0	0	6.5	0	0	5
			200	C	0	0	0	8	0	1
16193	71	*Sporobolus cryptandrus* (Torr.) A. Gray (sand drop-seed).	200	A	0.5	0	0.5	0	0	0
			200	B	1.5	0	6.5	0	0	3.5
			200	C	13.5	0	0	0	0	59
16194	70	*Sporobolus cryptandrus* (Torr.) A. Gray (sand drop-seed, hulled seed).	200	A	0	0	0	4.5	0	0
			200	B	0	0	0	7.5	0	20.5
			200	C	0	0	0	0	0	74.5
16195	10	*Triticum aestivum* L. (wheat)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16196	1	*Zea mays* L. (corn—Boone County White).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16197	2	*Zea mays* L. (sweetcorn—Early Concord).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
CYPERACEAE (SEDGE FAMILY)										
16198	74	*Cyperus esculentus* L. (yellow nut grass).	200	A	0	1.5	4.5	7	0.5	8.5
			200	B	0	2.5	5.5	21	5	5
			200	C	0	1.5	0	14	0	17
LILIACEAE (LILY FAMILY)										
16199	38	*Allium cepa* L. (onion)	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
CONVALLARIACEAE (LILY-OF-THE-VALLEY FAMILY)										
16200	16	*Asparagus officinalis* L. (asparagus)	100	A	0	0	1	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
MORACEAE (MULBERRY FAMILY)										
16201	17	*Cannabis sativa* L. (hemp)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
URTICACEAE (NETTLE FAMILY)										
16202	75	*Boehmeria nivea* (L.) Gaud. (ramie)	200	A	0	6	0	7	11.0	39
			200	B	0	43.5	0	10	32.0	61
			200	C	0	53.5	0	26	32.0	71
POLYGONACEAE (BUCKWHEAT FAMILY)										
16203	18	(*Fagopyrum fagopyrum* (L.) Karst. (buckwheat).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16204	40	*Polygonum pennsylvanicum* L. (smartweed).	200	A	0	0	0	0.5	4.0	0
			200	B	0	0	0	2.5	0	0
			200	C	0	0	1	1	0	0
16205	78	*Polygonum persicaria* L. (lady's-thumb, smartweed).	200	A	0	26	0	60.5	14	1
			200	B	0	1	0	31.5	9	25.5
			200	C	0	0	0	8	.5	55
16206	41	*Polygonum scandens* L. (climbing false buckwheat).	200	A	0	0	0	0	1	0
			200	B	0	0	0	0	0	2.5
			200	C	0	0	0	0	0	5
16207	76	*Rumex salicifolius* Weinm. (willow-leaved dock).	200	A	88.5	67	33	83	57.5	0
			200	B	85.5	75.5	19	62	83.5	72.5
			200	C	70.5	68	23	93.5	89	55
16208	39	*Rumex crispus* L. (curled dock) not cleaned.	200	A	67.5	69	63.5	60.5	42.5	9
			200	B	79.5	64.5	66.5	76	0	24
			200	C	79	58	50.5	72.5	19	13.5
16209	77	*Rumex obtusifolius* L. (broad-leaved dock, bitter dock).	200	A	73	91.5	88.5	82.5	56.5	59
			200	B	72.5	92	84	86	90	77.5
			200	C	79.5	93.5	86.5	89	83.5	82.5
CHENOPODIACEAE (GOOSEFOOT FAMILY)										
16210	81	*Axyris amaranthoides* L. (Russian pigweed).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16211	19	*Beta vulgaris* L. (sugar beet)	100	A	14	0	8	1	0	0
			100	B	39	10	35	1	0	1
			100	C	40	7	9	4	2	1
16212	79	*Chenopodium album* L. (lamb's-quarters, white goosefoot).	200	A	32	36.5	.5	39	30	0
			200	B	63.5	41	50.5	36.5	46.5	0
			200	C	64.5	51	.5	62	47	65.5
16213	80	*Chenopodium hybridum* L. (maple-leaved goosefoot).	200	A	7.5	14	1	36.5	0	0
			200	B	9.5	30	9	27	15	40.5
			200	C	13	34	41.5	39.5	11	0
AMARANTHACEAE (AMARANTH FAMILY)										
16214	82	*Amaranthus retroflexus* L. (rough pigweed).	200	A	9	0	1	11	0	0
			200	B	11	19.5	67.5	36	0	0
			200	C	17.5	12	10	48	0	0
PHYTOLACCACEAE (POKEWEED FAMILY)										
16215	42	*Phytolacca americana* L. (poke, pigeon berry).	200	A	7.5	65.5	29	87.5	0	[a] 0
			200	B	66.5	91	68.5	93	87.5	75
			200	C	80.5	93.5	77	77	93	72.5
PORTULACACEAE (PURSLANE FAMILY)										
16216	83	*Portulaca oleracea* L. (purslane)	200	A	39	15	36.5	21.5	12.5	8.5
			200	B	38.5	30.5	33.5	26.5	20	0
			200	C	30.5	39	11	6.5	14.5	38

[a] 34 hard seed recovered.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
SILENACEAE (PINK FAMILY)										
16217	43	*Agrostemma githago* L. (corn cockle).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16218	84	*Alsine media* L. (common chickweed).	200	A	90.5	64.5	2.5	5.5	0	0
			200	B	96.5	81	47	3	0	0
			200	C	92.5	69.5	50	21.5	0	0
16219	44	*Vaccaria vaccaria* (L.) Britton (cowherb)	100	A	0	0	0	0	0	0
			100	B	4	0	1	0	0	0
			100	C	7	0	1	28	0	0
BRASSICACEAE (MUSTARD FAMILY)										
16220	87	*Brassica nigra* (L.) Koch (black mustard).	200	A	5	27	0	1.5	0	0
			200	B	7	10.5	1	25	0	38
			200	C	7	9	0	25.5	0	.5
16221	45	*Brassica oleracea* L. (cabbage)	200	A	0	0	1	0	0	0
			200	B	0	0	.5	0	0	0
			200	C	0	0	0	0	0	0
16222	88	*Brassica campestris* L. (turnip)	200	A	0	0	0	0	0	0
			200	B	0	0	1	0	0	0
			200	C	.5	1.5	.5	3	0	0
16223	89	*Bursa bursa-pastoris* (L.) Britton (shepherd's purse).	200	A	0	6	0	2	0	0
			200	B	0	31	4.5	5.5	Missing.	0
			200	C	0	27.5	20	17	47	0
16224	47	*Erysimum cheiranthoides* L. (wormseed, treacle mustard).	200	A	1	0	1	0	0	0
			200	B	2.5	1	0	0	0	0
			200	C	4	0	.5	0	0	0
16225	46	*Neslia paniculata* (L.) Desv. (ball mustard).	200	A	23	3	0	9.5	0	0
			200	B	24.5	5.5	0	8	0	0
			200	C	38.5	10.5	2.5	14	0	0
16226	86	*Sisymbrium altissimum* L. (tall sisymbrium).	200	A	10.5	21.5	79	0	0	0
			200	B	17.5	17	6.5	2.5	0	0
			200	C	26	49	0	17	0	0
16227	85	*Thlaspi arvense* L. (field pennycress).	200	A	11	9.5	34	0	0	0
			200	B	8	62	52	46	0	.5
			200	C	11.5	31	7.5	10	0	0
ROSACEAE (ROSE FAMILY)										
16228	90	*Potentilla monspeliensis* L. (rough cinquefoil).	200	A	9.5	89.5	64	20.5	46.5	82.5
			200	B	16	91.5	63	97	Missing.	68.5
			200	C	21.5	95	54.5	88.5	59	91
CAESALPINIACEAE (SENNA FAMILY)										
16229	48	*Cassia marylandica* L. (wild senna, American senna).	100	A	3	9	14	14	3	1
			100	B	3	23	14	7	5	0
			100	C	5	25	15	14	3	2
FABACEAE (PEA FAMILY)										
16230	52	*Lespedeza frutescens* (L.) Britton (wand-like bush clover).	200	A	0	.5	0	4.5	0	[b]1
			200	B	0	1	.5	3	0	[c]2
			200	C	.5	1.5	1.5	2.5	0	[d]0
16231	49	*Medicago sativa* L. (alfalfa, lucern).	200	A	2	3.5	.5	0	0	0
			200	B	9	1	.5	0	0	0
			200	C	9	1	0	0	0	0
16232	4	*Phaseolus vulgaris* L. (bean)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0

[b] 15 per cent hard seed recovered. Germinated when clipped.
[c] 32 per cent hard seed recovered. Germinated when clipped.
[d] 48 per cent hard seed recovered. Germinated when clipped.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
FABACEAE (PEA FAMILY)—Continued										
16233	5	*Pisum sativum* L. (pea)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16234	51	*Robinia pseudacacia* L. (locust tree, false acacia).	200	A	0	5	4.5	21.5	19.5	[e])
			200	B	0	1	4.5	12.5	6	[f] 0
			200	C	0	3	4	18	2.5	[g] 0
16235	93	*Trifolium hybridum* L. (alsike clover).	200	A	2	1.5	0	3	2.5	2.5
			200	B	4	3	1	2	.5	4.5
			200	C	4.5	1.5	1.5	5.5	3.5	5
16236	50	*Trifolium pratense* L. (red clover).	200	A	1	0	0	1	.5	3.5
			200	B	2	0	0	.5	.5	3.5
			200	C	2	0	0	2.5	0	1
16237	91	*Trifolium pratense* L. (red clover) harvest, 1900.	200	A	4.5	2	.5	4	4	[h] 6
			200	B	5	2	1.5	9.5	Missing.	[h] 6
			200	C	6	5.5	1.5	32.5	1	[h] 2.5
16238	92	*Trifolium pratense* L. (red clover) hard seed from No. 16237.	200	A	10.5	4	4	1	15	[h] 15.5
			200	B	15.5	3	6.5	1.5	Missing.	[h] 15.5
			200	C	14.5	4.5	6	3.5	.5	[h] 9.5
16239	94	*Trifolium repens* L. (white clover).	200	A	0	0	0	3	2.5	[h] 0
			200	B	1	0	0	4.5	0	[h] .5
			200	C	0	0	.5	3	1.5	[h] 0
16240	3	*Vigna catjang* Walp. (iron cowpea).	100	A	0	0	0	0	0	0
			100	B	1	0	0	0	0	0
			100	C	0	0	0	0	0	0
LINACEAE (FLAX FAMILY)										
16241	53	*Linum usitatissimum* L. (flax, linseed).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
ANACARDIACEAE (SUMAC FAMILY)										
16242	20	*Rhus glabra* L. (scarlet sumac)	100	A	0	0	0	1	0	0
			100	B	0	0	0	0	0	0
			100	C	2	0	0	0	0	0
MALVACEAE (MALLOW FAMILY)										
16243	54	*Abutilon abutilon* L. (velvet leaf).	200	A	0	0	44.5	69.5	74.5	[i] 57
			200	B	0	0	24.5	77	77.5	[k] 39
			200	C	0	0	29.5	71.5	76	[l] 40.5
16244	6	*Gossypium hirsutum* L. (cotton)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16245	55	*Hibiscus militaris* L. (halberd-leaved rose mallow).	200	A	0	0	37.5	74	70	57.5
			200	B	0	0	66.5	80.5	58	36
			200	C	0	0	46.5	74	62.5	46.5
HYPERICACEAE (ST. JOHN'S WORT FAMILY)										
16246	95	*Ascyrum hypericoides* L. (St. Andrew's cross).	200	A	1	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	.5	0	0	0	0	0

[e] 27 per cent hard seed recovered. Germinated when clipped.
[f] 27.5 per cent hard seed recovered. Germinated when clipped.
[g] 31 per cent hard seed recovered. Germinated when clipped.
[h] Some hard seed recovered. Germinated when clipped.
[i] 12.5 per cent hard seed recovered. Germinated when clipped.
[k] 23 per cent hard seed recovered. Germinated when clipped.
[l] 18 per cent hard seed recovered. Germinated when clipped.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
ONAGRACEAE (EVENING PRIMROSE FAMILY)										
16247	96	*Onagra biennis* (L.) Scop. (common evening primrose).	200	A	0	48	0	74	57.5	61
			200	B	0	67	0	86	56.5	87.5
			200	C	0	70.5	0	89.5	45	86.5
APIACEAE (CARROT FAMILY)										
16248	57	*Apium graveolens* L. (celery)---	200	A	48.5	18.5	0.5	22.5	1.5	0
			200	B	64	23	3.5	15	1.5	10.5
			200	C	60	38	21	31.5	9	10.5
16249	56	*Pastinaca sativa* L. (parsnip, wild).	200	A	14.5	6	14.5	3	0	0
			200	B	25.5	6.5	11	3	0	0
			200	C	31.5	11	36	13.5	2.5	0
OLEACEAE (OLIVE FAMILY)										
16250	21	*Fraxinus americana* L. (white ash).	25	A	0	0	4	0	0	0
			25	B	0	0	12	0	0	0
			25	C	84	0	4	0	0	0
CONVOLVULACEAE (MORNING-GLORY FAMILY)										
16251	23	*Convolvulus sepium* L. (hedge bindweed, great bindweed).	100	A	2	29	11	41	47	27
			100	B	4	11	14	51	66	41
			100	C	7	13	21	43	64	43
16252	22	*Ipomoea lacunosa* L. (small-flowered white morning-glory).	100	A	20	80	68	22	6	7
			100	B	25	94	72	57	71	52
			100	C	33	88	92	83	2	57
CUSCUTACEAE (DODDER FAMILY)										
16253	98	*Cuscuta polygonorum* Engelm. (smartweed dodder).	200	A	11.5	10	1.5	16	Some.	[m] 25
			200	B	10.5	15.5	4	14.5	Some.	[m] 25
			200	C	13	16	3	9.5	Some.	[m] 25
16254	97	*Cuscuta epilinum* Weihe. (flax dodder).	200	A	15.5	0	0	0	0	0
			200	B	23.5	0	0	0	0	0
			200	C	34	0	0	8.5	0	0
VERBENACEAE (VERVAIN FAMILY)										
16255	100	*Verbena hastata* L. (blue vervain).	200	A	11.5	12.5	0	40.5	0	82.5
			200	B	13	20	0	44.5	0	71
			200	C	14	24	0	26	0	0
16256	99	*Verbena urticifolia* L. (white vervain, nettle-leaved vervain).	200	A	23.5	1	0	19	58	82.5
			200	B	24.5	4	0.5	24	0	90
			200	C	26.5	0	0	20.5	63.5	78
SOLANACEAE (POTATO FAMILY)										
16257	59	*Capsicum annuum* L. (red pepper).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0.5	0	0	0	0	0
16258	61	*Datura tatula*, L. (purple stramonium, jimson weed).	200	A	86	41	75.5	77.5	23.5	55
			200	B	84	37.5	98.5	95	82	78
			200	C	86.5	39.5	91	94.5	97.5	55.5
16259	60	*Lycopersicum lycopersicum* (L). Karst. (tomato).	200	A	0.5	0	0	0	0	0
			200	B	1	0	0	0.5	0	0
			200	C	0.5	0	0	0	0	0
16260	101	*Nicotiana tabacum* L. (tobacco).	200	A	46.5	0.5	28	39.5	19	46
			200	B	70	4.5	45	49.5	25.5	35.5
			200	C	55	18.5	44	78.5	28.5	56
16261	58	*Solanum nigrum* L. (black nightshade, garden nightshade).	200	A	9.5	76.5	31	2.5	0	81.5
			200	B	10.5	66.5	51.5	80.5	19.5	73.5
			200	C	12.5	62.5	27	90	28.5	94.5

[m] Estimated.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
SCROPHULARIACEAE (FIGWORT FAMILY)										
16262	102	*Verbascum thapsus* L. (great mullen).	200	A	7	17.5	11	63	37.5	86
			200	B	7.5	26	7.5	40.5	72	90
			200	C	25.5	29	2.5	82.5	32	92.5
PLANTAGINACEAE (PLANTAIN FAMILY)										
16263	105	*Plantago lanceolata* L. (ribwort, ribgrass, buckhorn).	200	A	20.5	33.5	19	3.5	0	0
			200	B	20.5	50.5	14	2.5	0	0
			200	C	20.5	62	17	4	1.5	0
16264	103	*Plantago major* L. (common plantain).	200	A	39.5	67.5	10.5	52.5	17.5	5.5
			200	B	43.5	59.5	10	32	30.5	13.5
			200	C	46.5	55	14.5	41	19.5	83.5
16265	104	*Plantago rugelii* Dec. (Rugel's plantain, broad plantain).	200	A	12	68	3.5	84	33	0
			200	B	12	55.5	5.5	74.5	29.5	20.5
			200	C	13.5	66	6	16	23.5	37
CUCURBITACEAE (GOURD FAMILY)										
16266	26	*Citrullus citrullus* (L.) Karst. (watermelon).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16267	25	*Cucumis melo* L. (muskmelon).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16268	24	*Cucumis sativus* L. (cucumber).	100	A	0	0	0	0	0	0
			100	B	1	0	0	0	0	0
			100	C	3	0	0	0	0	0
CICHORIACEAE (CHICORY FAMILY)										
16269	107	*Lactuca scariola* L. (prickly lettuce).	200	A	63.5	74.5	0	0	0	0
			200	B	69	59	0	0	0	0
			200	C	69.5	67	0	0	0	0
16270	62	*Lactuca sativa* L. (lettuce)	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0.5	0.5	0	0	0
16271	106	*Taraxacum erythrospermum* Andrz. (red-seeded dandelion).	200	A	35.5	29	6.5	0	0	0
			200	B	41.5	4.5	0.5	0	0	0
			200	C	45.5	1.5	8	0	0	0
AMBROSIACEAE (RAGWEED FAMILY)										
16272	63	*Ambrosia artemisiaefolia* L. (ragweed).	200	A	16	12.5	12	66	69	2
			200	B	18.5	11.5	6	69	69	83.5
			200	C	20.5	16	3.5	62	81	78.5
16273	28	*Ambrosia trifida* L. (great ragweed).	100	A	0	0	0	5	0	1
			100	B	2	9	0	13	12	15
			100	C	6	8	0	6	2	6
16274	27	*Xanthium pennsylvanicum* Walbr. (cocklebur).	[a] 20	A	0	0	0	5	15	0
			20	B	0	0	45	5	15	0
			20	C	5	0	25	[b]	0	0

[a] Ten fruits buried.

[b] Twenty-five sprouts recorded evidently an error, perhaps due to root sprouting.

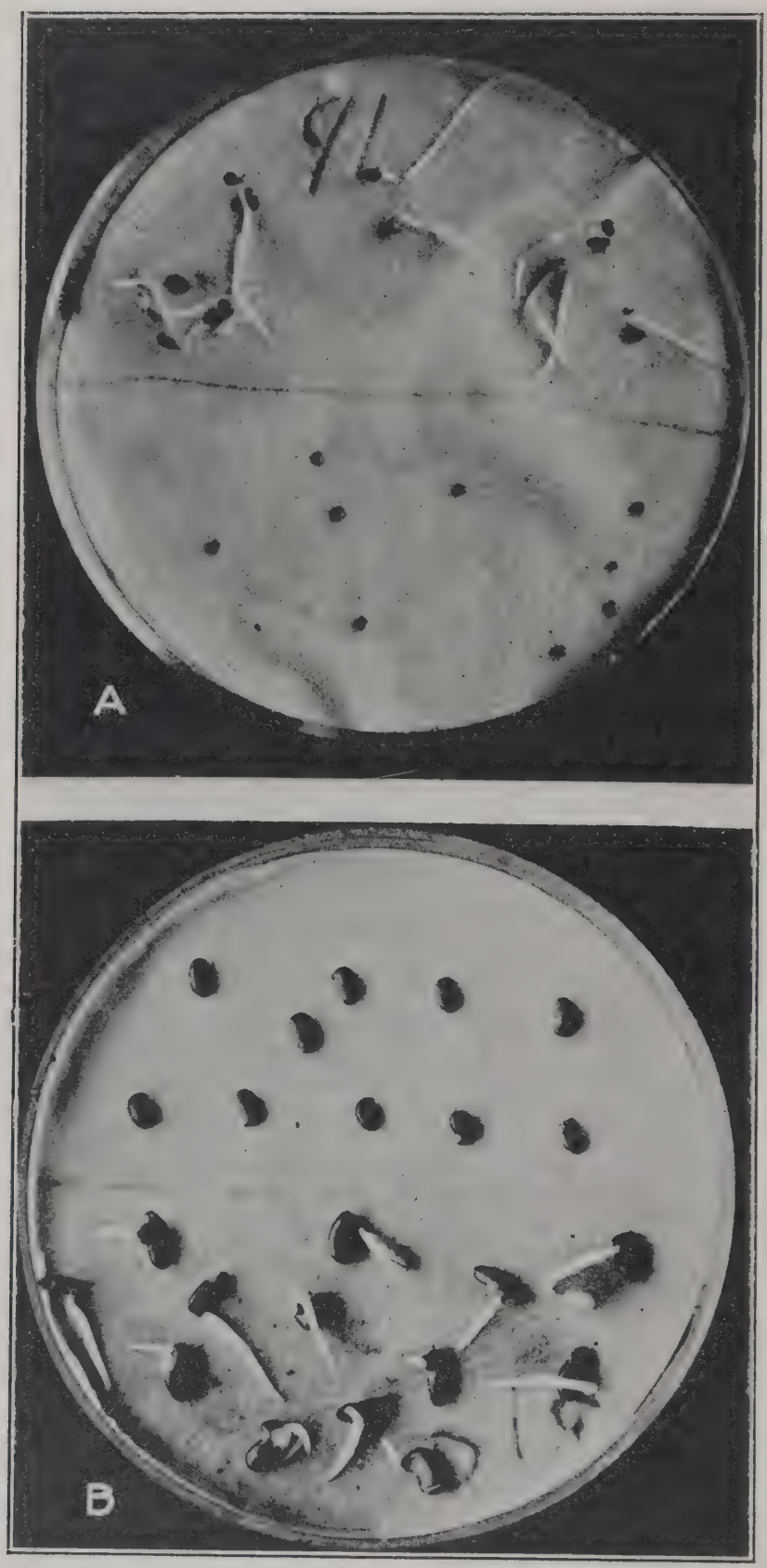
TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germiation in—					
					1903	1905	1908	1912	1918	1923
		ASTERACEAE (ASTER FAMILY)								
16275	112	*Arctium lappa* L. (burdock, clotbur).	200	A	42.5	31.5	12	53	10.5	0
			200	B	63.5	57.5	16.5	73.5	33.5	29
			200	C	73	54	25.5	93	67	17
16276	64	*Bidens frondosa* L. (black beggar ticks).	200	A	14.5	0	53	40	0.5	0
			200	B	16.5	0	38	63.5	0	0
			200	C	18	0	60.5	64.5	0	0
16277	111	*Carduus arvensis* L. (Canada thistle).	200	A	21	35	14.5	5.5	3	0.5
			200	B	22.5	28.5	15.5	9.5	0.5	0.5
			200	C	28.5	38.5	25.5	21	3	4.5
16278	110	*Chrysanthemum leucanthemum* L. (whiteweed, oxeye daisy).	200	A	21	78.5	47	78	37.5	42.5
			200	B	33	79	61.5	57	36.5	48
			200	C	49.5	76	81.5	82	20	39
16279	108	*Grindelia squarrosa* (Pursh) Dunal. (broad-leaved gum plant).	200	A	30.5	22	6.5	3.5	0	0
			200	B	36	10.5	1.5	0	0	0
			200	C	42	11.0	5	13.5	0	0
16280	29	*Helianthus annuus* L. (common sunflower, wild).	200	A	43.5	0	0	0	0	0
			200	B	64	0	0	0	0	0
			200	C	66.5	0	0	0	0	0
16281	7	*Helianthus annuus* L. (common sunflower, cultivated).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16282	65	*Onopordon acanthium* L. (cotton thistle, Scotch thistle).	200	A	86	0	82	39.5	36.5	37
			200	B	93	0	90.5	71.5	41.5	4
			200	C	90.5	0	87	61.5	31.5	7.5
16283	109	*Rudbeckia hirta* L. (black-eyed Susan).	200	A	6.5	35.5	1.5	52.5	20	30.5
			200	B	6.5	41	2	57.5	48.5	56.5
			200	C	7	58.5	2.5	66	10	52
		PINACEA (PINE FAMILY)								
16284	30	*Pinus virginiana* Mill. (scrub pine, Jersey pine).	100	A	0	0	0	1	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0

In 1912, two sets were taken up from each depth. One set was tested in the greenhouse and one in the laboratory germinating chambers. The higher germination in each case is included in Table I. Each time except in 1923 the seeds were taken up for test in the fall, usually just before the ground froze. In 1923, however, they were taken up in the early spring. At the close of the last test (1923) the soil in which the leguminous seeds were planted was examined for the presence of hard seeds. Red clover, white clover, black locust, and bush clover were found to have hard seeds remaining. Some of the hard seeds of each kind were clipped by scratching with emery paper. All so treated sprouted readily when subjected to conditions favorable for germination (Pl. 1).

Polygonum scandens produced sprouts for the first time in 1918. Both the hulled and unhulled seed of *Sporobolus cryptandrus* gave their highest germination in 1923. *Cuscuta polygonorum* was allowed to grow for identification and it became so tangled that an accurate count was impossible. The test of timothy was interrupted in both 1903 and 1905, which accounts for no sprouts being reported for the first two tests, although this seed grew in each of the later tests. In the case of *Xanthium pennsylvanicum* there were no sprouts in 1923, and an examination of the soil in the pots at the time of planting showed only one whole seed from the three depths, the remaining seeds being completely disintegrated.

Robinia pseudacacia produced no sprouts in the flats in 1923. Examina-



Vitality of Buried Seeds Plate 1

A.—*Trifolium pratense*, red clover. Clipped and unclipped seed.
B.—*Robinia pseudacacia*, locust tree. Clipped and unclipped seed.

tion of the soil from the three depths at the expiration of the test showed 27 per cent, 27½ per cent and 31 per cent of hard seed remaining.

For reference a list is appended of the species which produced sprouts in 1923, after being buried in the soil for 20 years. Twelve of these, marked *, have produced sprouts from each depth each year tested. Tobacco is the only cultivated crop included in the twelve.

**Chaetochloa verticillata* (foxtail).
Chaetochloa glauca (yellow foxtail).
Chaetochloa viridis (green foxtail).
Phalaris arundinacea (reed canary grass).
Phleum pratense (timothy).
Poa pratensis (Kentucky bluegrass).
Sporobolus airoides (hair-grass dropseed).
Sporobolus cryptandrus (sand dropseed).
Sporobolus cryptandrus (sand dropseed, hulled seed).
Cyperus esculentus (yellow nut-grass).
Boehmeria nivea (ramie.)
Polygonum persicaria (smartweed).
Polygonum scandens (false buckwheat).
Rumex salicifolius (willow-leaved dock).
Rumex crispus (curled dock).
**Rumex obtusifolius* (broad-leaved dock).
Beta vulgaris (sugar beet).
Chenopodium album (lamb's quarters).
Chenopodium hybridum (maple-leaved goosefoot).
Phytolacca americana (poke).
Portulaca oleracea (purslane).
Brassica nigra (black mustard).
Thlaspi arvense (field penny cress).
Potentilla monspeliensis (rough cinquefoil).
Cassia marylandica (wild senna).
Lespedeza frutescens (bush clover).
Robinia pseudacacia (black locust).
Trifolium hybridum (alsike clover).
Trifolium pratense (red clover).
Trifolium repens (white clover).
Abutilon abutilon (velvet leaf).
Hibiscus militaris (rose mallow).
Onagra biennis (evening primrose).
Apium graveolens (celery).
**Convolvulus sepium* (hedge bindweed).
**Ipomoea lacunosa* (white morning-glory).
Cuscuta polygonorum (smartweed dodder).
Verbena hastata (blue vervain).
Verbena urticifolia (white vervain).
**Datura tatula* (jimson weed).
**Nicotiana tabacum* (tobacco).
Solanum nigrum (black nightshade).
**Verbascum thapsus* (great mullen).
**Plantago major* (common plantain).
Plantago rugelii (broad plantain).
**Ambrosia artemisiaefolia* (ragweed).
Ambrosia trifida (great ragweed).
Arctium lappa (burdock).
**Carduus arvensis* (Canada thistle).
**Chrysanthemum leucanthemum* (oxeye daisy).
Onopordon acanthium (cotton thistle).
**Rudbeckia hirta* (black-eyed Susan).

Typical growth of some plants from the buried seed is shown in Plate 2.

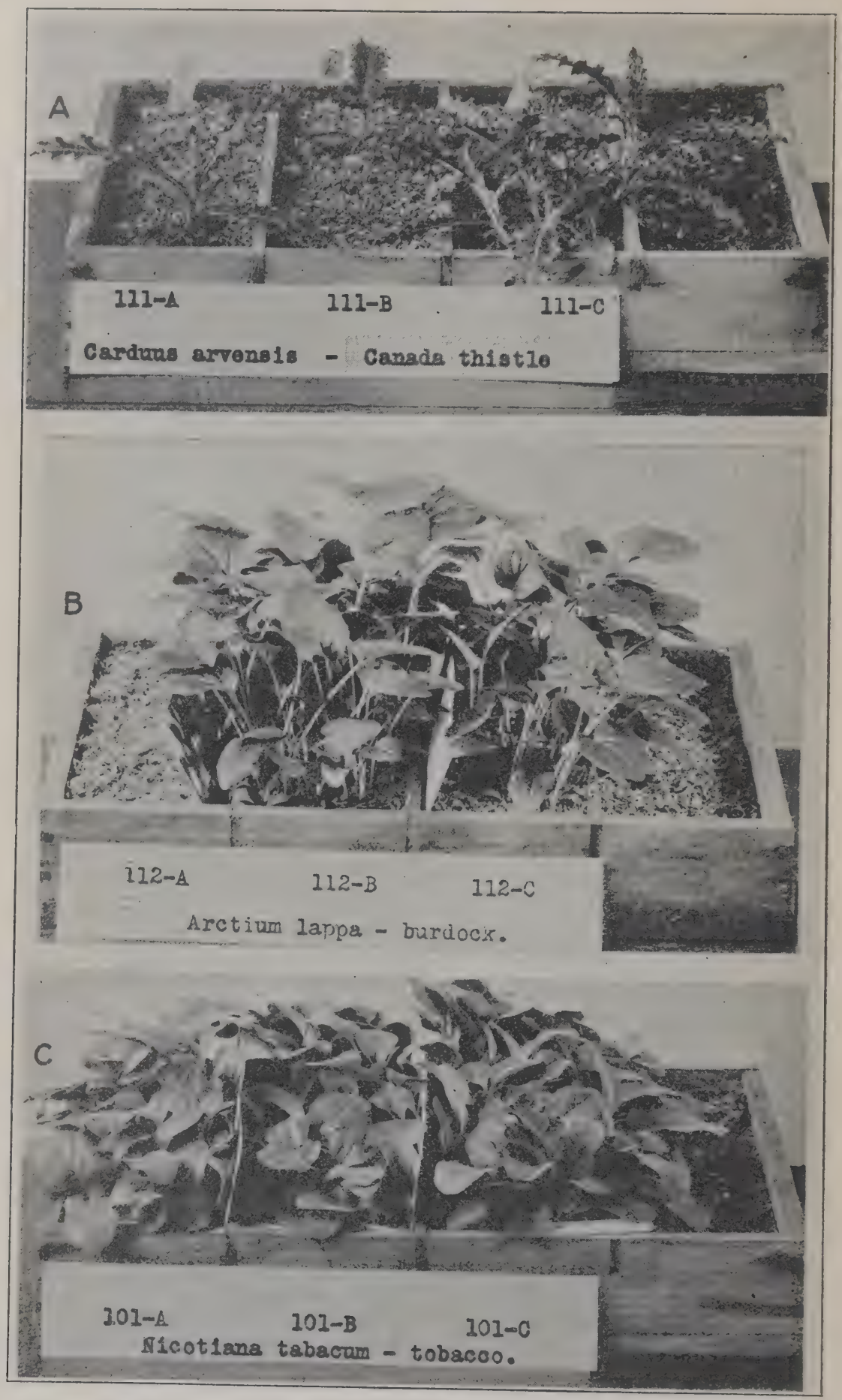
A comparison of the germination of all samples from the different depths shows that the seeds deteriorate somewhat more rapidly at the shallow or A depth. There is little difference between depths B and C. Taking the aggregate of all sprouts obtained in the six tests, 27 per cent came from depth A, 36 per cent from depth B and 37 per cent from depth C.

Of the total number of sprouts obtained in all years from all three depths, the proportion which grew in each year was: 18 per cent in 1903, 19 per cent in 1905, 15 per cent in 1908, 21 per cent in 1912, 12 per cent in 1918 and 15 per cent in 1923.

Table I shows that too much emphasis must not be placed on the actual germination percentage obtained. The results show that under the climatic and field conditions prevailing at Arlington farm, 51 of the 107 species lived 20 years buried in the soil; they do not, however, prove that the 56 species which failed to produce sprouts in 1923 were all dead. Table I shows several cases of growth in 1923 after failure in 1918; and *Polygonum scandens* grew for the first time in 1918. Later tests may bring out similar cases.

The vitality tests have been made as uniformly as possible under conditions which were thought to be best adapted to the majority of the species. However, we know little about the requirements for germination of many species. The variations in the results obtained from the same species in the different years and at different depths for the same year indicate that because of physiological changes in the seeds these requirements will vary with the seasonal and weather conditions prevailing previous to the viability test.

The following 15 species buried in Michigan by Doctor Beal were included by Doctor Duvel in this experiment: *Amaranthus retroflexus, Ambrosia artemisiaefolia, Brassica nigra, Bromus secalinus, Bursa bursa-pastoris, Agrostema githago, Onagra biennis, Plantago major, Portulaca oleracea, Rumex crispus, Chaetochloa glauca, Alsine media, Trifolium repens, Verbascum thapsus* and *Chenopodium al-*



A.—*Carduus arvensis*, Canada thistle.
B.—*Arctium lappa*, burdock.
C.—*Nicotiana tabacum*, tobacco.

bum. With the exception of *Trifolium repens* (white clover), which failed to produce any sprouts in Doctor Beal's experiment and which grew very sparingly every year but one in this experiment, the results check very closely. It might be expected that variations in soil and climate would influence the preservation of vitality of seeds buried in the soil; but too little information is available for comparison and definite conclusions.

Plowing under to exterminate weeds which have gone to seed does not accomplish its purpose. Each plowing of a field infested with weed seeds brings some of these seeds near enough to the surface to germinate, and at the same time buries others deeply enough to preserve their vitality. No normal crop rotation is long enough to effect eradication of persistent weeds.

When the experiment was started, seeds of 35 species were put in paper packages and the packages sealed in glass tubes. These tubes were buried at the deep depth. The seeds in one of these tubes, taken up in 1908, after being buried six years, were tested for germination both in the germinating chamber and in the greenhouse. The results obtained are given in Table II, together with the results obtained the same year from the same species taken from the soil in pots. In most cases the seeds from the tube gave a higher germination than those from the pots. The seeds of the cultivated plants gave distinctly better results from the sealed tubes than from the buried pots.

TABLE II.—*Germination obtained in 1908 in both laboratory and greenhouse from the samples of seeds in the sealed tube buried at depth "C" for six years, together with the percentage of sprouts obtained the same year from the same varieties of seed taken from the soil in pots*

Laboratory No.	Burial No.	Name of plant	Number of seeds	1908 percentage of germination of seeds from—				
				Soil in pots in greenhouse			Sealed tube	
				Depth A	Depth B	Depth C	In laboratory	In greenhouse
16179	33	*Chaetochloa glauca* (L.) Scribn. (yellow foxtail)	100	5.5	1.5	1.5	25	22
16180	67	*Chaetochloa virdis* (L.) Scribn. (green foxtail)	200	16	13	26.5	32	24
16187	32	*Panicum virgatum* L. (tall smooth panicum)	100	0	0	0	35	3
16188	34	*Phalaris arundinacea* L. (reed canary grass)	200	29.5	52.5	38	12.5	0
16192	69	*Sporobolus airoides* Torr. (hair-grass drop-seed)	200	0	6.5	0	69.75	7
16193	71	*Sporobolus cryptandrus* (Torr.) A. Gray (sand drop-seed).	200	0.5	6.5	0	0	5
16204	40	*Polygonum pennsylvanicum* L. (Pennsylvania persicaria).	200	0	0	1	2	0
16207	76	*Rumex salicifolius* Weinm. (willow-leaved dock).	200	33	19	23	[illegible]	46
16210	81	*Axyris amaranthoides* L. (Russian pigweed)	200	0	0	0	0.5	0
16212	79	*Chenopodium album* L. (lamb's quarters, white goosefoot).	200	0.5	50.5	0.5	55.5	30
16213	80	*Chenopodium hybridum* L. (maple-leaved goosefoot).	200	1	9	41.5	67	28
16214	82	*Amaranthus retroflexus* L. (rough pigweed)	200	1	67.5	10	94.25	19
16215	42	*Phytolacca americana* L. (poke, pigeon berry)	200	29	68.5	77	86	38
16216	83	*Portulaca oleracea* L. (purslane, pussley)	200	36.5	33.5	11	89.5	9.5
16219	44	*Vaccaria vaccaria* (L.) Britton (cowherb)	200	0	1	1	84	12
16220	87	*Brassica nigra* (L.) Koch (black mustard)	200	0	1	0	0	12.5
16223	89	*Bursa bursa-pastoris* (L.) Britton (shepherd's purse).	200	0	4.5	20	49.25	19
16227	85	*Thlaspi arvense* L. (field pennycress)	200	34	52	7.5	25.5	53
16228	90	*Potentilla monspeliensis* L. (rough cinquefoil)	200	64	63	54.5	52	15
16231	49	*Medicago sativa* L. (alfalfa, lucern)	200	0.5	0.5	0	89.5	73
16235	93	*Trifolium hybridum* L. (alsike clover)	200	0	1	1.5	76.5	55
16236	50	*Trifolium pratense* L. (red clover)	200	0	0	0	85	67
16239	94	*Trifolium repens* L. (white clover)	200	0	0	0.5	84	66
16241	53	*Linum usitatissimum* L. (flax, linseed)	200	0	0	0	90.5	83
16247	96	*Onagra biennis* (L.) Scop. (common evening primrose).	200	0	0	0	22	0.5
16248	57	*Apium graveolens* L. (celery)	200	0.5	3.5	21	72.25	58
16253	98	*Cuscuta polygonorum* Englem. (smartweed dodder).	200	1.5	4	3	15.25	13
16255	100	*Verbena hastata* L. (blue vervain)	200	0	0	0	0	0
16260	101	*Nicotiana tabacum* L. (tobacco)	200	28	45	44	89.5	43
16264	103	*Plantago major* L. (common plantain)	200	10.5	10	14.5	99	20.5
16265	104	*Plantago rugelii* D c. (Rugel's plantain, broad plantain).	200	3.5	5.5	6	69.5	0.5
16270	62	*Lactuca sativa* L. (lettuce)	200	0	0	0.5	98	76
16279	108	*Grindelia squarrosa* (Pursh) Dunal., (broad-leaved gum plant).	200	6.5	1.5	5	85.5	36.5
16283	109	*Rudbeckia hirta* L. (black-eyed Susan)	200	1.5	2	2.5	59.25	13.5
16254	97	*Cuscuta epilinum* Weihe. (flax dodder)	200	0	0	0	0	0

SUMMARY

The depth at which the seeds were buried had little effect upon the preservation of their vitality.

Cultivated plants appear to depend largely upon human agencies for their perpetuation.

None of the cereals or legumes whose seeds are used as food germinated on being dug up.

The seeds of weeds or wild plants survived better than those of cultivated plants.

The weed seeds showing the highest germination and the fewest failures were all from common and persistent weeds in the locality of Arlington, Va. The docks, lambs' quarters, plantains, daisies, poke, purslane, jimson, and ragweed are examples.

Of the 107 species buried in 1902, 71 grew in 1903 after 1 year, 61 grew in 1905 after 3 years, 68 grew in 1908 after 6 years, 69 grew in 1912 after 10 years, 50 grew in 1918 after 16 years, and 51 grew in 1923 after 20 years.

The seeds of most weeds, when ploughed under, will not perish during the period of any normal crop rotation.

Any attempt to control weeds which have gone to seed by plowing them under is evidently futile. This conclusion does not invalidate the importance of plowing weeds under before they go to seed.

The preservation of seeds buried in the soil helps to provide a continuous vegetative cover for the land.

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POSTNATAL GROWTH OF THE BODY, SYSTEMS, AND ORGANS OF THE SINGLE-COMB WHITE LEGHORN CHICKEN[1]

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INTRODUCTION

The problem of growth has been studied in many forms of life and from various points of view, and yet many of its fundamental aspects are still unknown or imperfectly understood. A great amount of data has been accumulated concerning the growth of mammals, but comparatively little concerning the lower forms of life. In regard to birds, there have been a few studies of the growth in body weight of the domestic fowl, but little attention has been given to the growth of the individual organs and systems. A systematic study of growth has therefore seemedd esirable, for various reasons. The domestic fowl (*Gallus domesticus*) is easily obtained, is omnivorous, and has a relatively short period of growth. Its economic value adds to the importance of a better knowledge of its anatomy and of the growth changes occurring in its various organs and systems during its development.[2]

REVIEW OF LITERATURE

The literature upon the growth of the domestic fowl consists chiefly of a few reports, giving the average weight of a number of birds at certain stages of their growth, and usually covering only brief periods.

Petrov (*19*)[3] studied the effects of hunger on the body weight in fowls, including a few observations upon a group of normal controls at various ages. He does not state the breed used and his data are, therefore, of little value for comparison. Weiske (*30*) studied the growth of 11 chickens from 6 days of age to 1 year. He later autopsied a newly hatched chicken of the same breed. He killed and autopsied individual specimens at intervals throughout the year and recorded the weights of the fresh and oven-dried skeletons and the feathers, immediately after removal from the chicken and again after oven-drying. He further studied the chemical composition of the feathers and skeletons.

Houssay (*7*) studied the growth of a number of chickens and plotted the growth curves. Stefanowska (*23*) plotted separate growth curves of growth in body weight for the males and females of a group of chickens.

Minot (*15*, *16*) followed the growth of two male and eight female chickens, making weighings daily at first and later less frequently up to 190 days; then three weighings between 335 and 350 days of age. He found an initial decrease in weight after hatching, similar to the postnatal decrease in mammals. Lee (*13*) made a very careful study of the fattening of poultry. The time during which the maximum gain occurs, the amount of feed required, and the average gain for several different breeds were determined. Mitchell and Grindley (*17*) made a study of the same problem for poultry as well as for some of the larger farm animals. Philips (*20*) published the results of growth experiments during four years upon

[1] Received for publication Mar. 11, 1924—issued January, 1925. This work was done cooperatively by the Department of Anatomy of the University of Minnesota and the Divisions of Poultry Husbandry and Veterinary Medicine of the Minnesota Agricultural Experiment Station.

[2] This study was undertaken upon the suggestion of Prof. C. M. Jackson and Prof. R. E. Scammon, of the University of Minnesota, to whom the writer wishes to express the deepest gratitude for counsel and advice during the course of the investigations. The writer is also greatly indebted to Prof. A. C. Smith and Prof. C. P. Fitch, of the University of Minnesota, for the generous provision of material and facilities for study.

[3] Reference is made by number (italic) to "Literature cited," p. 396–397.

several thousand single-comb White Leghorn chicks at the Indiana Experiment Station. The sexes were weighed together until the young cockerels could be picked out, and from this time on to the end of the twenty-fourth week only the pullets were weighed. Card and Kirkpatrick (*2*) published their growth studies which had been carried on during the three preceding seasons. They used single-comb White Leghorns and Rhode Island Reds from the regular stock of the Connecticut Experiment Station at Storrs, Conn. The chicks were carefully weighed in lots, once per week, and the average weight per chick determined for 24 weeks. The cockerels were removed at the end of the eighth week and after this only the pullets were weighed. Buckner, Wilkins, and Kastle (*1*) studied the growth in body weight of two lots of single-comb White Leghorn chickens at the Kentucky Experiment Station. One group was incubator-hatched and raised in a brooder; the other was hatched and reared by hens. They started with 60 chicks in each lot. The chickens were weighed individually each week and the average for each sex was determined for a period of 28 weeks.

When we turn to the growth of the systems or organs of the domestic fowl, we find still less in the literature. Welcker and Brandt (*28*) give the body weight and the weights of the organs and systems in two male domestic fowls. There are also some weights given for the organs of the chick on the 9th, 10th, 11th, 13th, 17th, 20th and 21st days of incubation, one "Junges Hühnchen vom Markte" and a hen. The breeds are not given and, of course, the number of cases is not adequate to determine the average weights. They give similar data for nine other species of birds, including two pigeons and two domestic geese. Zaitschek (*31*) autopsied "131 Stück voll entwickelten Hühnern ungarischer Rasse und verschiedenen Alters." He finds that the blood (that which escaped from the chicken) forms an average of 3.8 per cent (range 2.6 to 5.6 per cent); the feathers average 7.7 per cent (range from 1.4 to 11.4 per cent); and the liver forms an average of 2.9 per cent (range from 1.4 to 4.7 per cent) of the live weight. These were the only organs weighed separately. Stieve (*25*) studied the development of the ovary in the hen, but made no observations upon the other organs.

MATERIAL AND METHODS

The chickens used were single-comb White Leghorns, which were provided by the Division of Poultry Husbandry. They were hatched in four groups. Group 1 was hatched May 29, 1920, but the weighings were not begun until June 26, at which time there were 95 chicks four weeks old. On July 13 this lot was transferred to two coops with free range, and 32 culls were removed. On October 27, 1920, they were moved into winter quarters and the sexes were separated. Group 2 was hatched July 8, 1920, and included 21 chicks. Group 3, hatched July 17, 1920, consisted of 36 chicks. Groups 2 and 3 were kept in the brooder house, but during the latter part of the summer and fall they were allowed to run in a large, grassy, fenced lot adjacent to the small runs. The last chick in Group 2 was autopsied November 13, 1920. Groups 1 and 3 were in part carried through to the end of the experiment, and the remaining chickens returned to the Poultry Department. The males and females in Group 3 were not separated. Group 4 included 18 chicks, hatched August 11, 1920. The last one of this group was autopsied November 15, 1920.

All of the chicks were hatched in the incubators at the poultry plant and, with the exception of Group 4, they were all put into the brooder house as soon as all chicks in the incubator were dry. This was usually about 24 hours after the first chick had hatched. The age of the chickens was counted from the time of removal from the incubator, so there is a variation of a maximum of about 24 hours in their ages. Group 4 was given to an old hen confined in a coop. This allowed free range for the chicks. Later the hen and chicks were put in a section of the brooder house with access to a grassy run.

From the beginning, all were fed a commercially prepared mixture of seeds, and a commercially prepared mash. After June 21, 1920, all the chicks were provided with hoppers filled with a mash composed of equal parts, by weight, of bran, hominy, middlings, ground oats, and beef scraps. As soon as old enough, they were given a mixture of cracked grains, corn, oats, etc., and later whole grain. The last three groups were given milk to drink from the beginning, and Group 1 was also given milk after June 26. After November 27, 1920, the remaining

chickens were given the regular "laying mash," hopper fed. This mash consists of the following ingredients: 6 lbs. corn meal, 4 lbs. ground oats, 4 lbs. middlings, 2 lbs. bran, 2 lbs. alfalfa meal, 7 lbs. beef scraps, ¼ lb. charcoal, 1 per cent common salt, 3 per cent bone meal. With this mash was given the usual allowance of grain. Throughout the experiment the chickens were fed by the Poultry Division, so that they might have the usual care and diet.

Complete autopsies were made on 100 normal chickens, 50 from Group 1, 15 from each of Groups 2 and 3, and 14 from Group 4. In addition to these 94 normal chicks at various ages, 6 older normal chickens (adults) were likewise autopsied for comparison.

The number of birds used is not sufficient to establish final conclusions on many points; but the results will at least indicate the general trend of the growth changes which occur in the single-comb White Leghorn from time of hatching to maturity. These results will serve as a basis for further and more detailed study of various phases of the problem, such as the variations due to nutrition and other environmental factors, variations in different breeds of fowls, etc.

WEIGHINGS.—The chickens in Group 1 were weighed once a week from June 26, 1920, to March 4, 1921. The other three groups were weighed every day from day of hatching, or when they were removed from the incubator, until the 1st of October; then only three times per week until December 3, and once per week thereafter, until killed and autopsied. The weighings were always made in the morning before the chickens were fed or allowed the freedom of the yard or range. They were weighed on a pair of spring milk-scales, sensitive to a hundredth of a pound. The entire group, or as many as possible, were weighed at a time, and the average weight per chick (in grams) was determined.

AUTOPSIES.—In selecting individuals for autopsy those which were most nearly in accord with the weights as given by Card and Kirkpatrick (2) at corresponding ages were selected at various periods. This makes the average weight of the autopsied chickens higher than the average live weight of the entire lot at the corresponding points (fig. 1).

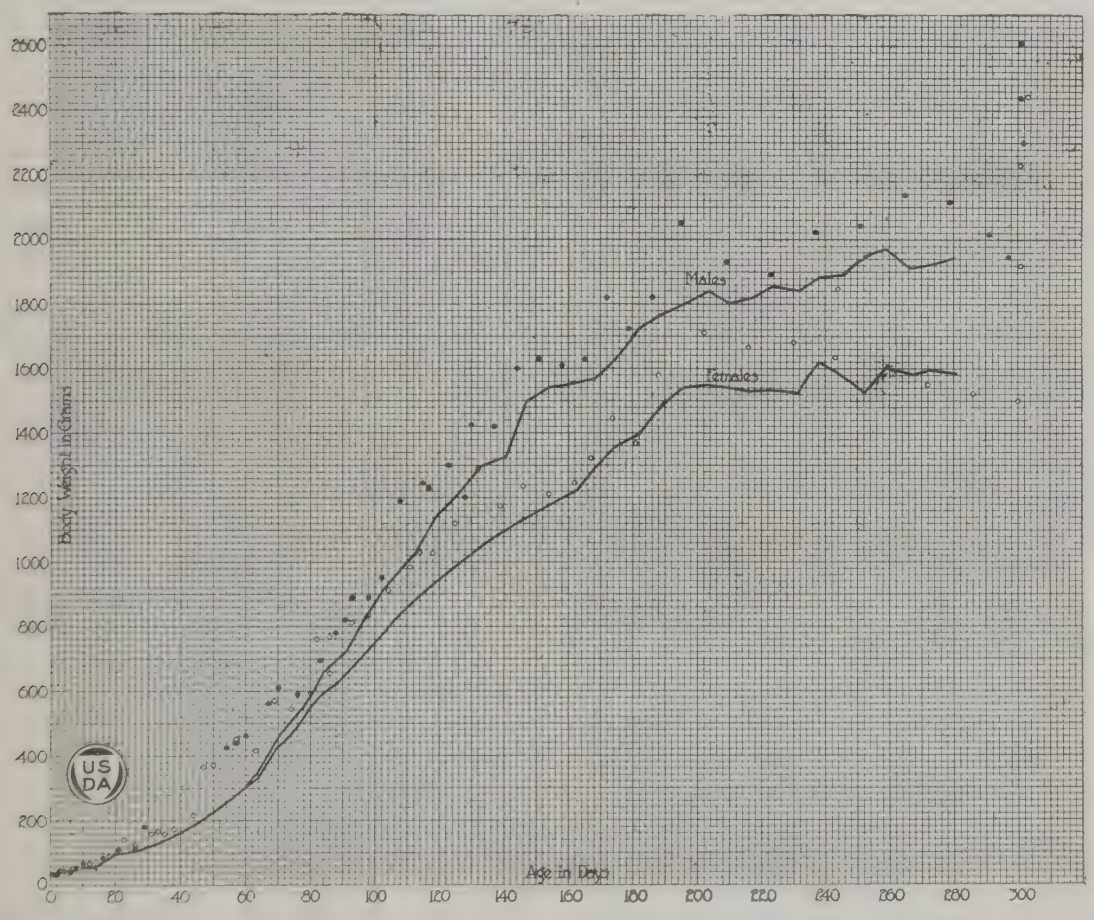

FIG. 1.—The two point-to-point curves show the average observed live weight per chicken for all groups combined. Up to the 56th day the line represents the average for both sexes; after this time the upper line represents the average for the males and the lower line that for the females. The six entries just beyond the 300-day line are the six adults (of uncertain age)

The technic of the autopsy was as follows: The chickens were chloroformed and then weighed. The feathers were pulled off and the chicken again weighed, the difference giving the weight of the feathers. The measurements of the chick were made after the removal of the feathers and the second weighing. The bird was laid right side down on a piece of paper, the neck gently straightened and the length from the tip of bill to anus marked on the paper. The distance from back to toe was measured in a similar manner. The other measurements were made with a pair of dividers. They include the following: Length of left leg from tip of toe to greater trochanter; length of left wing, from tip to proximal end of humerus; length of head; dorsoventral and transverse diameters of the thorax, just behind the anterior end of the sternal crest.

The head was then cut off, the incision being made close to the base of the skull and through the spinal cord as it passes through the foramen magnum. The esophagus and trachea were severed just below the pharynx.

The blood was allowed to drain from the severed vessels and was not measured. The blood, larger masses of fat, mesenteries and larger vessels and nerves were not weighed separately. The integument was next removed. This includes the skin, with scaly skin from legs and feet, horny beak and claws; also some dermal fat which could not easily be removed, and the dermal muscles, especially in the neck and lateral thoracic regions. The uropygial glands were removed with the skin. The wattles and ear lobes were not weighed separately at first, and so are included in the integument of the smaller chicks. They were not of sufficient size to modify the results, for as soon as it was possible to separate them they were weighed separately.

So far as possible, all fat and mesenteries were removed from the viscera, and they and the parts of the body were immediately put in a moist chamber until weighed. The organs were weighed in closed containers on a chemical balance sensitive to one-tenth of a milligram. The body, after removal of all viscera, was cleared of all excess fat, mesenteries, etc., and weighed, in the case of the larger chickens, on a laboratory balance sensitive to one-tenth of a gram.

The digestive tube includes all of the canal from beginning of esophagus to termination of intestine in the cloaca. The cloaca was not included. The entire digestive tube, together with contents, was first weighed; then the stomach and gizzard were cut out, opened, and each weighed after removal of contents. The crop was opened and contents removed. Next by gentle pressure the contents of esophagus and intestines were forced out. Then esophagus, crop, and intestines were weighed together. From these weighings the weight of the entire empty canal and the "tare", weight of contents, were computed.

The muscles were then removed by careful dissection, and the ligamentous skeleton, with contained central nervous system, was weighed. Then the brain and the spinal cord were removed and weighed.

The data were recorded upon individual record cards and finally plotted in the form of the various graphs and curves shown in Figures 1 to 31, inclusive.

The curves shown in Figure 1 are point-to-point curves made by connecting the average body weight of the chickens for each week. In the construction of the other curves the numerical averages of the gross body weight and the absolute weight of the organ were determined in general for each increase of 200 grams in gross body weight, and these average points were plotted on the preliminary chart. Then by means of French curves the growth curves were drawn in to fit the group averages as closely as possible. Later an empirical formula was determined for each curve and the curves as shown on the final charts were drawn according to the formulas. The final charts show the weights or measurements of the individual chickens as dots and circles and the growth formula empirically determined is shown by the heavier line. The percentage weights were plotted on the same abscissae as the absolute weights of the same organ. Double-weighted medians were determined for these values as for the weights in grams, as described above. Then by means of French curves a curve was drawn through the double-weighted medians. This curve, without individual percentage values, is shown as the lighter line on the final charts. The net body weight (gross body weight minus the weight of the contents of the digestive tube) was used in determining the percentage weights. Crelle's Rechentafeln was used in computing the data.

GROWTH OF THE BODY AND PARTS

GROWTH IN BODY WEIGHT

Growth in body weight is but an incidental and subordinate part of the problem. The increase in the gross body weight of the White Leghorn chick has been worked out thoroughly and for a large number of chicks by Philips (*20*), Card and Kirkpatrick (*2*), Buckner (*1*), and others. The weighing of the chicks for the present experiment was undertaken chiefly to serve as a check upon the individuals selected for the autopsies. It will be shown, however, that the various groups in the present experiment give some information on the relative effects of certain kinds of food and care upon growth of the chickens. A reduction of 23 days in the period between hatching and egg production (as in Group 3 compared with Group 1), is a matter of practical interest. The importance of the time of hatching is also indicated by the slow growth of Group 4, a late-hatched group.

Figure 1 [4] shows the three phases of the postnatal growth of the entire chicken, which are as follows: A period of relatively slow increase in weight; a period of rapid growth; a decrease after the time of sexual maturity is reached, with the resulting flattening (horizontal tendency) of the curve. The irregularities in the terminal portion of the curve (especially noticeable in Figure 1) are due in part to the much smaller number of specimens weighed in the later periods. Any individual fluctuation consequently would modify the curve to a much greater degree.

The growth curves of the individual groups were plotted but not shown with the accompanying charts. The slow growth of Group 4 was very noticeable. In Groups 1 and 3 there is a slight flattening of the curve for the females at about 150 days for Group 1, and 130 days for Group 3, or about 40 days before egg-laying began. This would suggest a prepuberal pause in growth, which does not occur in other domestic animals. The significance of this pause is questionable.

The growth data as given by the previous investigators, when plotted on the same scale as the present figures, are of interest for comparison. The data of Philips (*20*) and Card and Kirkpatrick (*2*) include only the weights of the females after it is possible to separate the males. Buckner and others (*1*) give the data for males and females separately. For comparison, the data for the two sexes are here grouped together up to the ninth week. When the growth curves are compared it is seen that the average weight of the chickens used in the present work falls below the other three at first. It never runs so high as the curve constructed from the data of Card and Kirkpatrick. They carried their weighings only through the twenty-fourth week, but at this time the chickens (females only) averaged 1,489.12 gm. while the Minnesota chickens averaged 1,298.44 gm. The chickens reported by Philips (*20*) from the Indiana Experiment Station were heavier than those discussed in the present paper, up to the thirteenth week, when both groups averaged about 650 gm., but from this time on they were a very little lighter. The Indiana experiment was continued for only 24 weeks, at which time the chickens averaged 1,248.50 gm., while the writer's averaged 1,298.44 gm.

The curve constructed from the data on the growth of the chickens reported by Buckner and others (*1*) from the Kentucky Experiment Station is more irregular than any of the others. It also runs higher than the present series up to between the thirteenth and fourteenth weeks, then it falls below. At the end of the 28 weeks the pullets from the Kentucky station averaged 1,447.5 gm. for the hen hatched and reared, and 1,120.4 gm. for the artificially hatched and reared pullets, as compared with 1,539.06 gm. for the artificially raised Minnesota pullets. The Minnesota cockerels likewise average lighter than both the artificially and the hen-raised Kentucky cockerels up to the nineteenth and twentieth weeks. After this time the cockerels of the present investigation were heavier than the Kentucky artificially

[4] KEY TO FIGURES

SOLID DOT.—Weight or measurement of the males (except in figure 27).
CIRCLE.—Weight or measurement of the females (except in figure 27).
ADULT.—Adult chickens (the three older cockerels and the three two-year-old hens).
HEAVIER LINES.—Curves of growth of absolute weight (measured in grams) or measurements (in centimeters), all drawn according to formula, except Figure 1.
LIGHTER LINES.—Curves of relative weight or percentage of the net body weight. Individual cases not shown.
Y (ORDINATES).—(On left margin) weight of the part in grams or length in centimeters; (on the right margin) percentage of the net body weight.
X (ABSCISSAE).—Gross body weight in grams, or age in days.

raised cockerels and slightly heavier than the hen-raised males. At the end of the twenty-eighth week, when their experiment was concluded, the Minnesota cockerels averaged 1,802.38 gm.; the hen-hatched and hen-reared cockerels from the Kentucky Station averaged 1,748.1 gm.; and the artificially hatched and reared averaged 1,594.6 gm.

At hatching, the average weight for the series of this investigation is 36.59 gm.; for the Connecticut chicks, 36.70 gm.; and for the Kentucky series 41.5 gm. for the hen-hatched and 41.6 gm. for the artificially hatched.

not been kept continually before it. When milk and a constant supply of mash were supplied, Group 1 began to improve. The greater freedom of the range was given to this group alone, yet it lagged behind the other groups and the first egg was laid by this group at 189 days, and by Group 3 at 166 days. Thus Group 3 began laying at an age of 23 days younger than Group 1.

The chart in Figure 2 gives only the first 21 days of the curve of Figure 1, plotted on a larger scale. The formula from which this curve was drawn is: $Y=[0.1(X+1)^{1.03}-0.104X-0.026]453.59$, in which Y represents the gross body

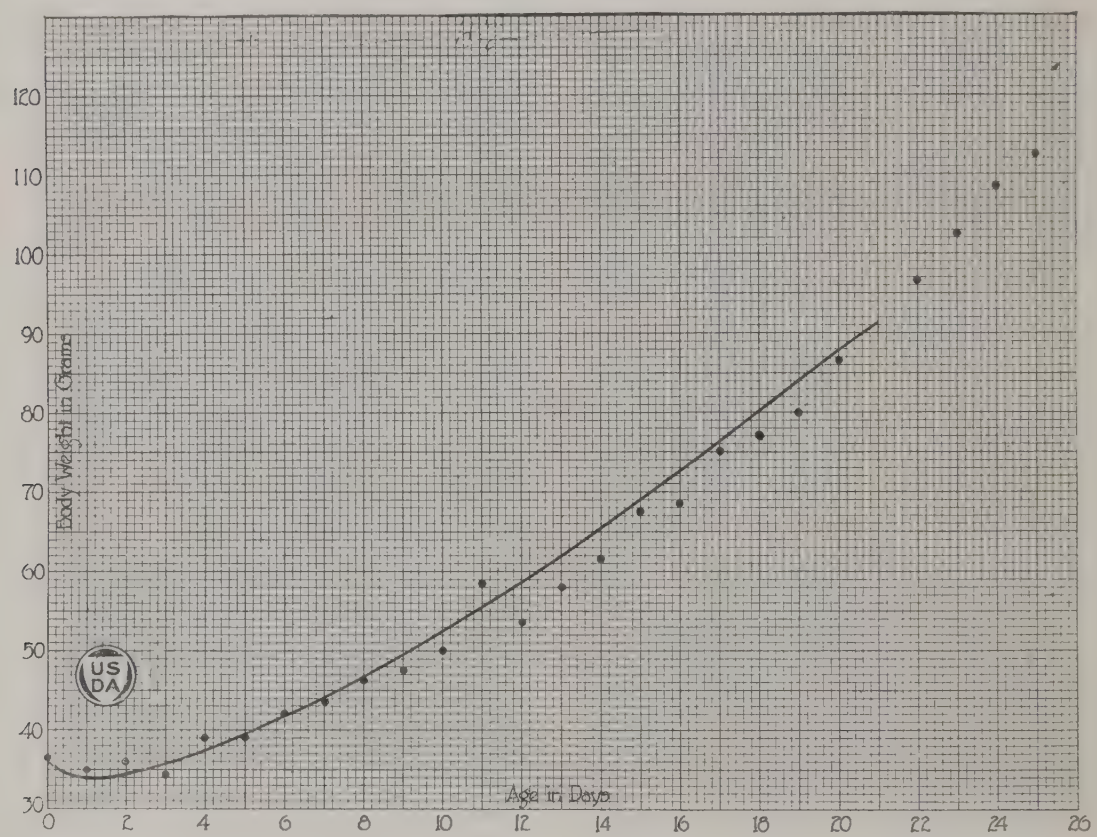

FIG. 2.—Average weight in grams of all chicks for the first 21 days. This curve is plotted on a larger scale to show the postnatal decrease in body weight

There is a difference in the growth of the chicks in the four groups here discussed. At the time of removal from the incubator their average weights were: Group 2, 34.47 gm.; Group 3, 36.29 gm.; and Group 4, 39.01 gm. Group 1 was not weighed until the chicks were 28 days old, and at that time the respective weights were: Group 1, 87.8 gm.; Group 2, 136.1 gm.; Group 3, 129.3 gm.; and Group 4, 113.4 gm.

Group 4, it should be remembered, was hatched late in the summer, and although it was hen-reared, which has been shown to be better than artificial brooding, this did not compensate for the unfavorable weather conditions. Group 1, up to this time (28 days) had been given no milk, and dry mash had

weight of the individual chick and X represents the age in days.

The computed weight drops to 33.5 gm. on the first day, while the observed average was 35.2 gm.; so a correction of about 1.7 gm. must be made in computing the weight of the one-day-old chick. The numerical averages for the chicks were: Day of hatching, 36.43 gm.; first day, 35.2 gm.; second day, 36.24 gm.; third day, 34.5 gm.; fourth day, 39.2 gm.

This curve shows the characteristic postnatal decrease in weight, which is found in so many animals and which Minot (*15*, *16*) found to persist in the chick until "by the fourth or fifth day they appear to entirely recover." It will be seen that the average weight of

the chicks from Groups 2, 3, and 4 is below the initial weight on the first, second, and third days, with the minimum on the third day. The initial weight is that observed when they were taken from the incubator. Not until the fourth day is the average weight greater than the initial weight. This decrease takes place although the chicks begin picking up sand as soon as placed in the brooder. The gizzard of the chick autopsied after one day was filled with fine sand, and the digestive-tract contents formed 4 per cent of the net body weight. In the chick at two days this tare formed 12.1 per cent, and at three days it had risen to 15.8 per cent of the net body weight.

The gross weights of the 100 autopsied chickens shown in Figure 1 are higher than the average live weights for the groups from which they came. During the earlier part of the work, individuals conforming to the average weight as given by Card and Kirkpatrick (*2*) for corresponding ages were selected, and this naturally raises the average for the autopsied chickens.

The drop in the average weights of the pullets after 200 days (fig. 1) is similarly due to the removal of the larger pullets earlier in the experiment, and is not the result of a decrease in weight of the individual pullets. The weights of the six older birds indicate a continued slow average increase in the period after the weighings were stopped. This increase is due very largely to an increase in adipose tissue, although some organs continue to grow, as will be shown later.

From the foregoing data it may be concluded that the chicks show a postnatal loss in body weight which is not recovered until the fourth day. The curve of postnatal growth in weight shows three general phases: First a period of slow growth, then a period of rapid growth, followed by a slow increase which may continue for some time. There is possibly a slight prepuberal retardation and rise, but this is doubtful. After 70 days the cockerels become distinctly heavier than the pullets of corresponding age.

GROWTH IN LINEAR MEASUREMENTS

BODY LENGTH.—The growth in length of the chick from the tip of the beak to the cloacal orifice is shown in the chart in Figure 3. The formulas

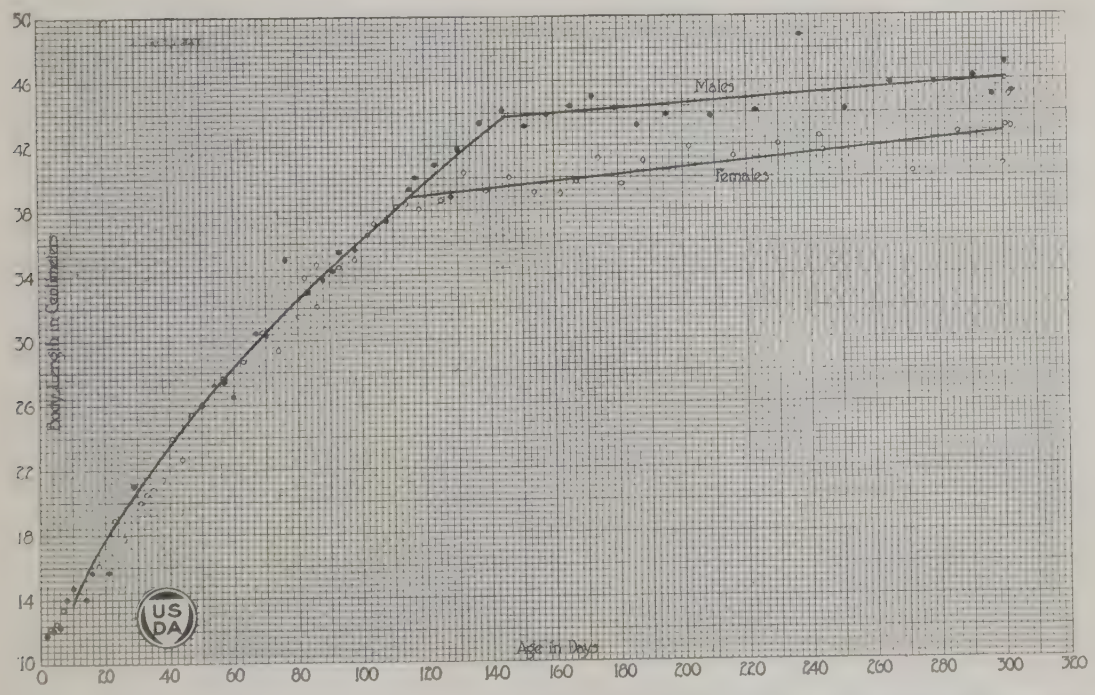

FIG. 3.—Nose-to-anus length, or body length from tip of bill to cloacal opening, measured in centimeters and plotted on age in days. The females (circles) appear relatively shorter after 115 days of age

from which these curves were drawn are, for the males from 10 to 145 days:

$$Y=2(X^{0.58})+0.013X+6.07;$$ from 145 to 300 days, $Y=0.0143(X-145)+43.79$.

The formulas are, for the females from 10 to 115 days:

$$Y=2(X^{0.58})+0.013X+6.07,$$ and from 115 to 300 days, $Y=0.0211(X-115)+38.89$.

Y represents the body length in centimeters and X represents the age in days.

A comparison of this chart and that in Figure 1 shows that nearly the maximum length is reached at about 115 days for the pullets, or when they reach about 55 per cent of the mature weight;

and about 30 days later for the cockerels, or when they reach about 75 per cent of their mature weight. The second part of the curve for both male and female is a straight line, but not a horizontal line as in the following linear measurements. This may be explained in part by the fact that this measurement is not entirely a skeletal measurement because it is affected by the distention of the abdomen. The greater increase in body weight in the pullets after completion of ossification is in part due to the additional weight of the reproductive tract and the greater amount of fat. Still these two factors will not account for all the difference. We must, therefore, conclude that the maximum length, or the fusion of the epiphyses and diaphyses occurs earlier in the pullet, not only in relation to age but also in relation to the attainment of the total body weight.

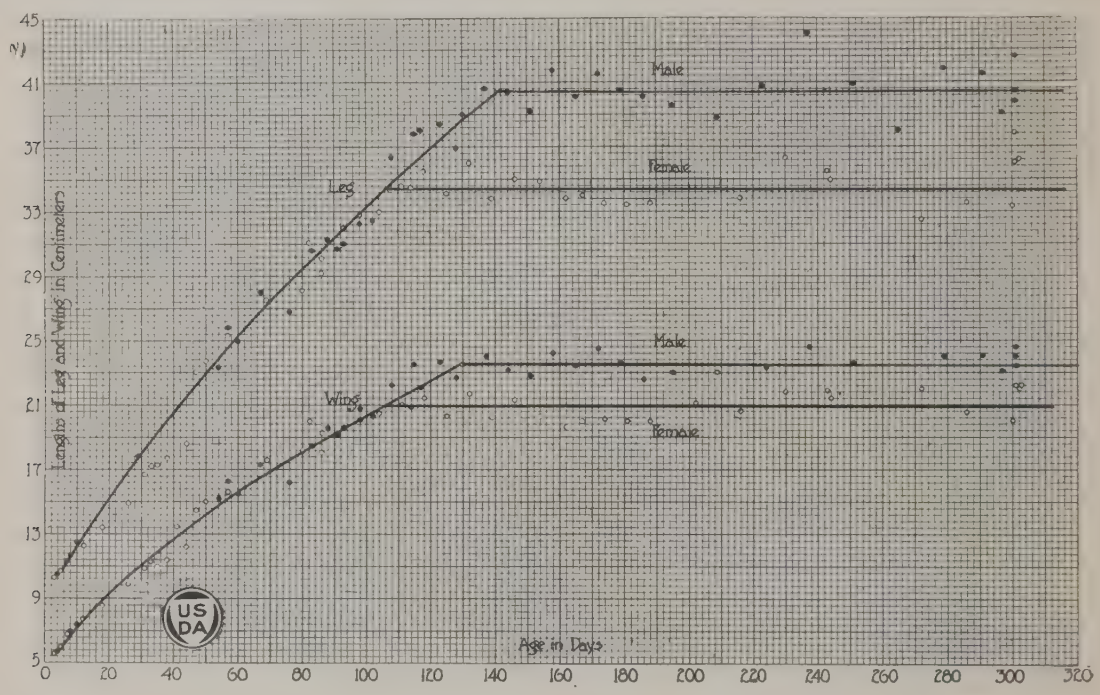

FIG 4.—The upper set of curves and individual cases show the length of the leg (in centimeters) from the greater trochanter of the femur to the tip of the extended middle toe. The length of wing from the greater tuberosity of the humerus to the tip of extended wing is shown in the lower part of the chart

EXTREMITIES.—Figure 4 shows the increase in length of the leg in the upper curves and of the wing in the lower curves. The formula from which the curves for leg length were drawn is:

$$Y = X^{0.75} - 0.05X + 6.68$$

in which Y represents length of leg in centimeters and X represents age in days. For the males the formula applies from 5 to 141 days. The curve then remains constant at 40.5 cm. The formula was used in drawing the curve for the females from the fifth to the one hundred and sixth days, after which time it remains constant at 34.40 cm. The back-toe measurements were also plotted; but the curve corresponded closely to that for the leg (except that it was about 1.5 cm. greater) and it has, therefore, been omitted from the charts. The left leg and left wing only were measured.

The growth in length of the leg corresponds closely with that of the body, the completion of growth (curves becoming nearly horizontal) being at nearly the same time for each. The males become taller, however, and continue to increase in height after the females have attained their maximum height. The growth of the length of the leg is practically completed at 141 days in the males and at 106 days in the females. In this chart the average leg length for the mature males is 40.5 cm. and 34.4 cm. for the mature females. In all the charts of the linear measurements the "adults" are larger, but they were selected from their groups as heavier chickens and consequently would be apt to have a larger skeleton and be larger in other respects.

The lower curve in Figure 4 shows for the wing length a less marked sex difference. The formula for the curves of wing length is:

$$Y = X^{0.58} + 0.028X + 3.12$$

in which Y represents length of wing in centimeters and X represents age in days. The formula was used for the males from 5 to 130 days, after which the curve remains constant at

23.57 cm. For the females the formula applies from 5 to 105 days and then the curve remains constant at 20.92 cm. In this case the time of the cessation of growth in length is also less definitely marked. The adult wing length in the male is reached at 130 days, or 11 days earlier than the adult leg length, and at 105 days in the female, which is about the same time that the maximum leg length is attained. Thus the pullets show an earlier development in both these measurements.

THORAX.—In Figure 5 the two diameters of the thorax are plotted against age. The formula from which the curves for the dorsoventral diameter were drawn is:

$$Y = X^{0.45} + 0.014X + 0.44$$

in which Y represents the dorsoventral diameter in centimeters and X represents age in days. For the males this formula applies from 10 to 150 days and then the curve remains constant at 12.06 cm. For the females the curve was drawn from the formula from 10 to 125 days, after which this curve remains constant at 10.97 cm. The formula for the transverse diameter of the thorax is: $Y = X^{0.37}$, in which Y represents the transverse diameter of the thorax measured in centimeters, and X represents age in days. This formula applies from 10 days of age to the adult stage. The dorsoventral diameter was taken just back of the anterior end of the crest of the sternum and the transverse diameter was always taken in the same plane. Both of these diameters show a longer period of increase than do the preceding linear measurements. This may be explained by the fact that these measurements are not purely skeletal. As can be seen by a comparison of the two sets of curves, the transverse diameter increases for a much longer time than does the dorsoventral diameter. This measurement is affected more by the deposit of fat, a large mass of subdermal fat being found on either side of the breast in the fat chickens. Another factor which influences these measurements is the possible increase in size of the thoracic cavity and the dilation of the bony thorax. This measurement and the body length are the only two which continue to increase throughout the entire series and both of them involve other factors than skeletal growth. The six older chickens differ from the others more in these two measurements than they do for the preceding measurements shown in Figures 3 and 4.

The thoracic index

$$\frac{\text{dorsoventral diameter} \times 100}{\text{transverse diameter}}$$

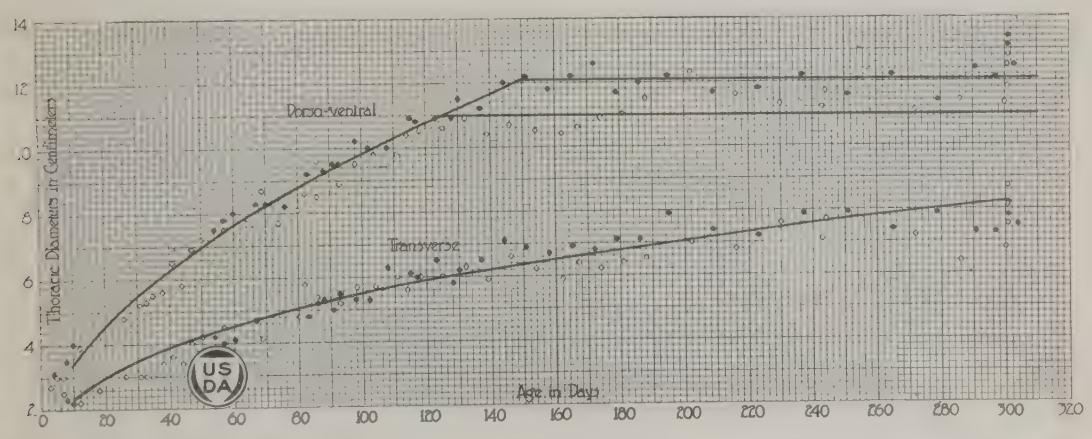

FIG. 5.—Dorsoventral diameter (upper part of chart) and transverse diameter of the thorax (lower part of chart) plotted on age in days. Both diameters (in centimeters) were made just posterior to the anterior end of the sternum. The dorsoventral diameter appears smaller in the older females

was determined for the chickens. Throughout the entire series, and including the six older birds, there appears no significant change and no sex difference. The index averages about 175.

Had these two diameters of the body been taken in the abdominal region there would have been apparent a much more noticeable sex difference; for in the later part of the period during which the birds were studied there is a marked difference in the shape of the body in the male and female. In the male the maximum depth or dorsoventral diameter is but a centimeter or two caudad to the point used in measuring this diameter. In the pullets, however, the anterior end of the body had the smaller dorsoventral diameter. In other words, the posterior region of the female body cavity becomes distended with the larger reproductive tract, and the greater

accumulation of fat in this region, tending to push downwards the ventral body wall. This produces the ventral sagging of the abdominal wall, which is characteristic of a laying hen.

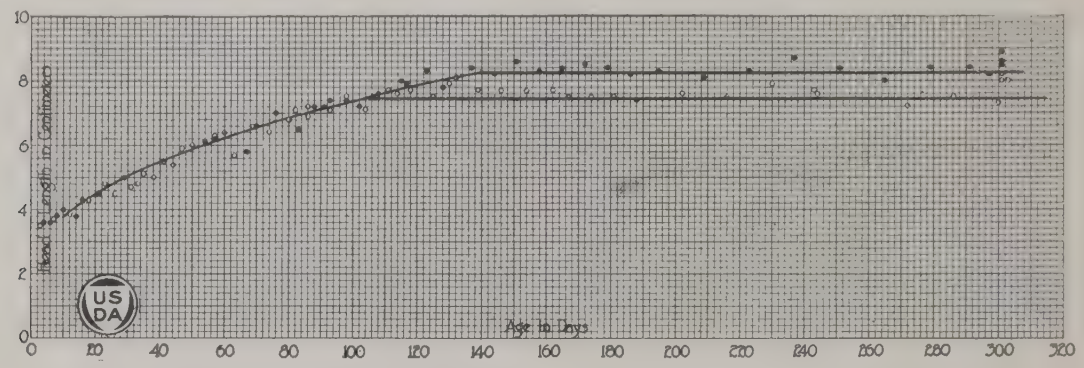

FIG. 6.—Length of head (in centimeters) from tip of bill to the most posterior part of the head, plotted on age in days. After about 105 days the head appears shorter in the females

HEAD LENGTH.—Figure 6 shows the increase in length of the head. The formula is: $Y = X^{0.36} + 0.007X + 1.44$ in which Y represents the head length in centimeters and X represents the age in days. The formula applies to the males from 10 to 140 days, then the curve is constant at 8.34 cm. For the females the formula applies from 10 to 105 days after which time the curve remains constant at 7.5 cm. The growth curve of the head length resembles the wing length more closely than the leg length. There is less sex difference than would be expected from the weights. As will be shown later, the male comb is far larger and the weight of the head in the older male birds is consequently greater than the weight of the head in the females. A study of Figures 6, 7, and 10 will suggest that the sex difference so evident in the head weight is due very largely to the greater weight of the male comb and wattles. The linear measurements when plotted on age all show a continuous convexity superiorly, although the curves show different rates of growth and rates differing at different ages of the chicks. The linear measurements employed

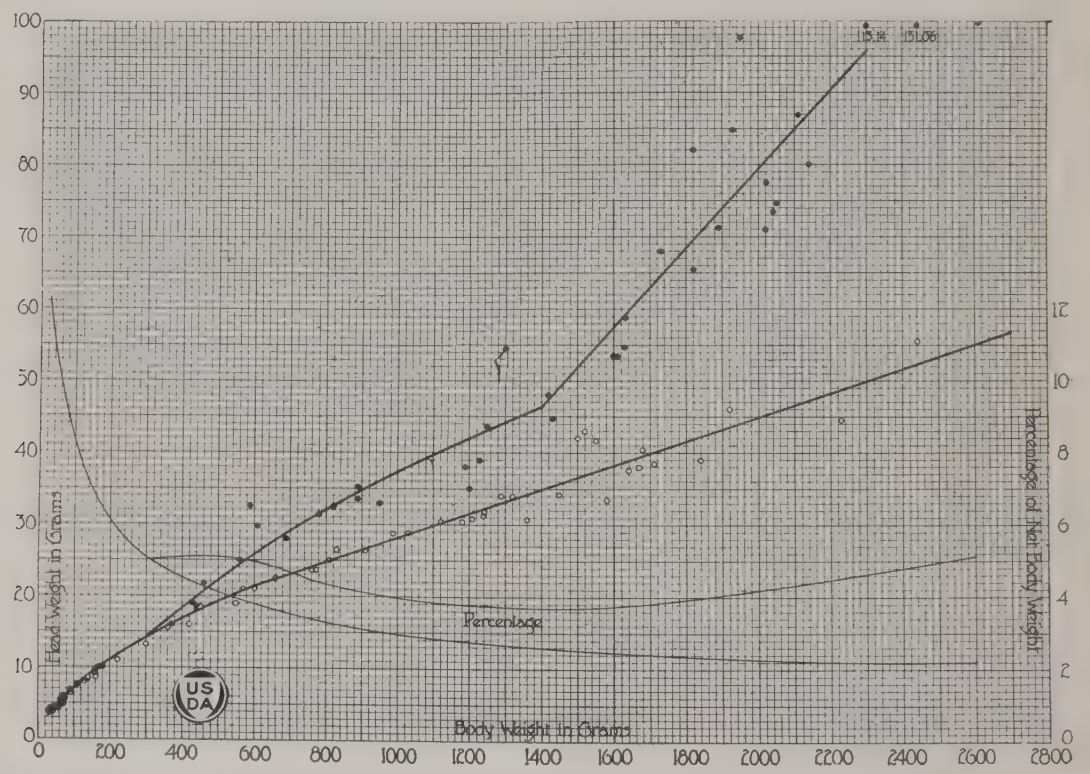

FIG. 7.—Growth in absolute and percentage weight of the entire head, without feathers, plotted on gross body weight. The individual specimens at 2,300 and 2,440 gm. gross body weight were too heavy to be included on the chart. A sex difference in head weight appears beyond about 300 gm. gross body weight. This difference is accentuated by the increased growth of comb in males above 1,400 gm. in gross body weight

all show an earlier maximum than do the curves of gross body weight (fig. 1), doubtless due to complete ossification of the skeleton before adult weight is attained.

HEAD WEIGHT.—The increase in absolute weight of the head plotted on gross body weight and the percentage of the net body weight are shown in Figure 7. The formulas used in drawing these curves of growth in absolute weight of the heads of the males are:

$$Y = X^{0.5} - 3$$

from 50 to 300 gm. gross body weight,

$$Y = X^{0.6} - 0.013X - 12.72$$

from 400 to 1,400 gm. gross body weight, and,

$$Y = 0.0554\ (X - 1400) + 46.28$$

from 1,400 to 2,300 gm. gross body weight.

The formulas for the females are:

$$Y = X^{0.5} - 3$$

from 50 to 600 gm. gross body weight,

$$Y = 0.0167\ (X - 600) + 21.49$$

from 600 to 2,500 gm. gross body weight.

In all these formulas Y represents the weight of the head in grams and X represents the gross body weight in grams. The cases in which the body weight is over 2,200 gm. are the adult specimens. They are not included in the curve, although the individual head weights are indicated on the chart. From 300 gm. gross body weight, or 55 days of age, there is a sex difference in head weight, due in large part, as suggested above, to the greater development of the comb and wattles in the males (fig. 10). The percentage weight of the head shows no initial rise but drops rapidly at first and later shows a sex difference. Jackson and Lowrey (*9*) found that in the postnatal growth of the rat the head at first increases more rapidly than the rest of the body and there is no sex difference. The head is relatively much lighter in the chicken, for its maximum percentage weight in the chicken is a little less than half that for the rat. In the adult rat the head forms about 9 per cent of the body, while in the chicken it is only about 4 per cent in the male and between 2 and 3 per cent for the female.

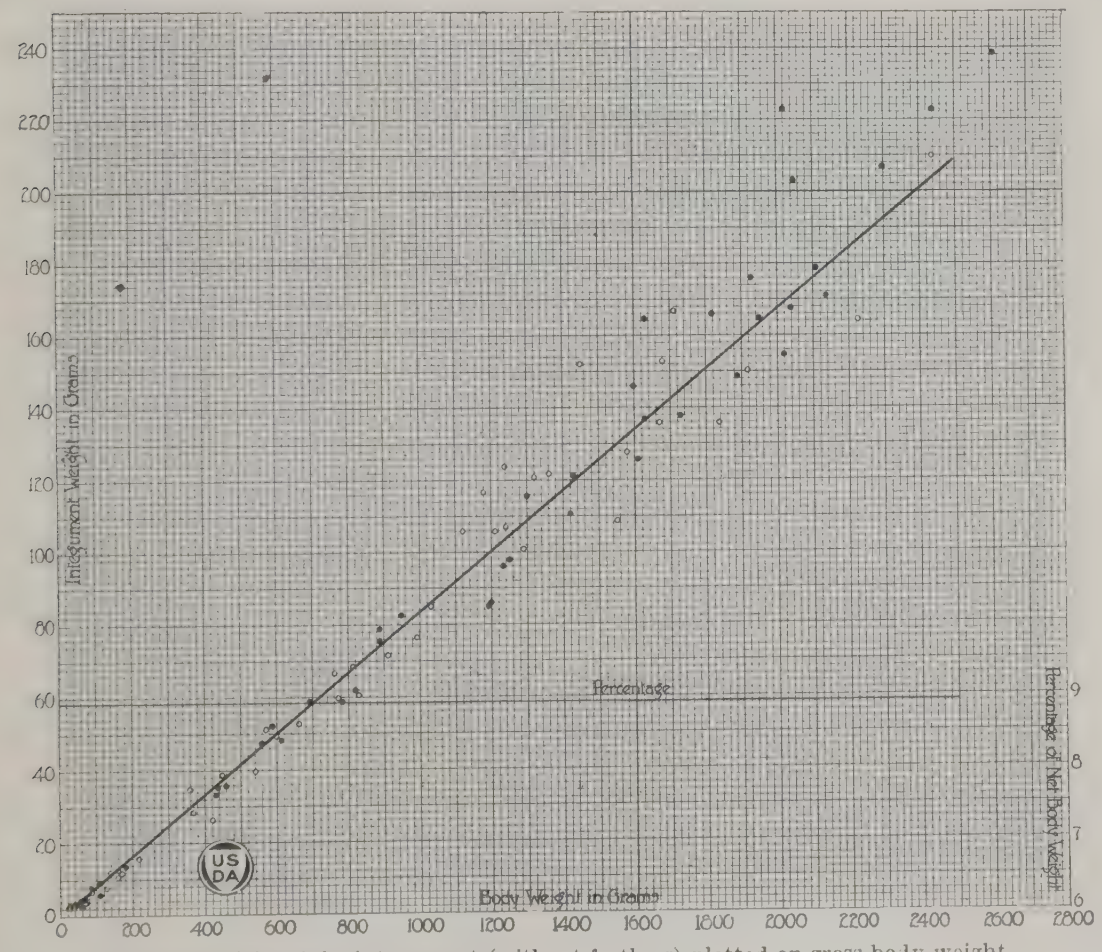

FIG. 8.—Weight of the integument (without feathers) plotted on gross body weight

GROWTH OF THE SYSTEMS AND ORGANS

INTEGUMENT

SKIN.—Figure 8 shows the weight of the integument, excluding feathers,

comb, and wattles, plotted on gross body weight, also its percentage of the net body weight. The formula is:

$$Y=0.0836X$$

in which Y represents the weight of the integument in grams and X the gross body weight in grams.

From this chart it is clearly evident that the growth of the skin is in direct proportion to the total increase in body weight, and that there is no appreciable sex difference. The percentage weights of the skin show no marked changes, forming about 8.9 per cent of the net body weight. This is less than the figures given by Welcker and Brandt (*28*), who give 18.86 per cent for the skin and subdermal fat for the domestic fowl, and from 12.57 to 18.07 per cent for the skin only of the other species of birds investigated. Their percentage weights doubtless include the feathers, however, which would make them more nearly comparable with those in the charts. The percentage weights of the skin for the various animals as given by Welcker and Brandt show a wide range and are in most cases greater than the values found in the Minnesota chickens (without feathers). According to Wiedersheim (*29*), the skin of birds is relatively thin; moreover, in removing the feathers, the shaft is not left in the skin to be weighed with the integument.

FEATHERS.—The growth of the feathers (fig. 9) follows a course entirely different from that of any of the preceding curves. To some extent it resembles the growth of the thymus (fig. 22).

The formulas for the males are:

$$Y=(0.01X)^{1.4}+0.0545X-0.45$$

from 100–600 gm. gross body weight,

$$Y=0.1\ (X-600)+44.53$$

from 600–1,640 gm. gross body weight,

$$Y=148.53-0.04\ (X-1,640)$$

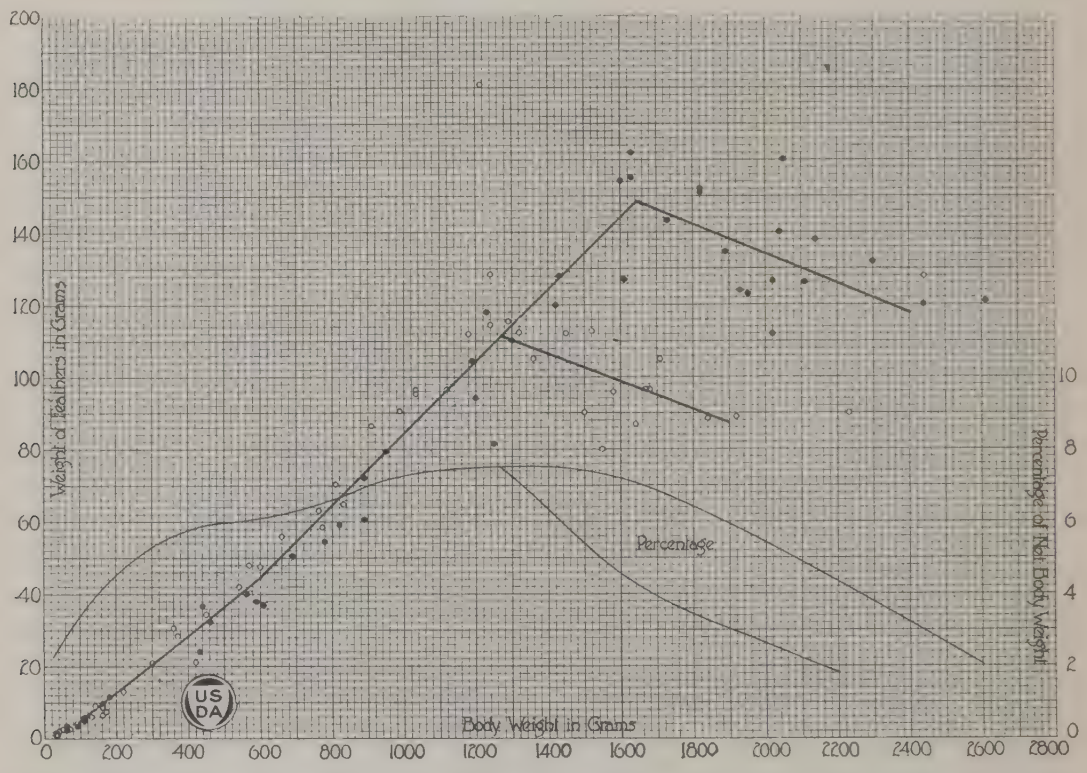

FIG. 9.—Weight in grams and percentage weight of the feathers. The abscissa represents the gross body weight. The case at 1,210 gm. and all of those above 2,200 gm. gross body weight are shown on the chart though not included in the data for the formula. A sex difference is apparent in the larger specimens

The formulas for the females are:

$$Y=(0.01X)^{1.4}+0.0545X-0.45$$

from 100–600 gm. gross body weight,

$$Y=0.1\ (X-600)+44.53$$

from 600–1,270 gm. gross body weight,

$$Y=111.53-0.038\ (X-1,270)$$

from 1,270–1,900 gm. gross body weight.

In all these formulas Y represents the weight of the feathers in grams and X represents the gross body weight in grams.

In single-comb White Leghorns the wing feathers begin to appear very early and the rest of the plumage is developed earlier than in some other breeds. The weight of the feathers increases more rapidly than the body weight, increasing from a little over 4.5 per cent to nearly 8 per cent of the net body weight, and then decreasing again.

After 1,260 gm. gross body weight there is a sex difference in the percentage weights. The absolute weight of the feathers increases without any sex difference until the body weight reaches 1,270 gm.; then the curve for the females begins to decline. The curve for the male plumage increases up to a gross body weight of 1,640 gm. and then it too decreases. The sex plumage, the sickle feathers, saddle feathers and the long feathers on the neck are the last to develop, and the growth of these probably carries the curve for the male plumage to a higher level than that reached by the female plumage. The same rise, followed by a decrease in the female and later in the male plumage, is also observed when the absolute weights are plotted against age.

This rise and fall in both relative and absolute weights, plotted against both the gross body weight and against time, are correlated with changes in the structure of the feathers. Until the feather is completely developed, the shaft contains a large amount of vascular tissue, but later the vascular tissue in the shaft atrophies and dries out. This results in a marked decrease in weight of the plumage. The female plumage is completed before that of the male, thus resembling the growth of the body as a whole and the ossification of the skeleton, as already shown.

Weiske (*30*) reports an increase followed by a decrease in the absolute and relative weights of the feathers in his 12 specimens. In the newly hatched chick weighing 40.9 gm. the weight of the feathers is given as 0.7295 gm. The maximum weight given is 83.59 gm. at 34 weeks when the gross body weight is 1,094 gm. From this point the weight of the feathers decreases to 57.23 gm. at one year.

The percentage values rise from 3.07 per cent at hatching to a maximum of 14.61 per cent at 660 gm. gross body weight (twenty-fourth week) and again decrease to 6.86 per cent at one year of age or a gross body weight of 1,360 gm.

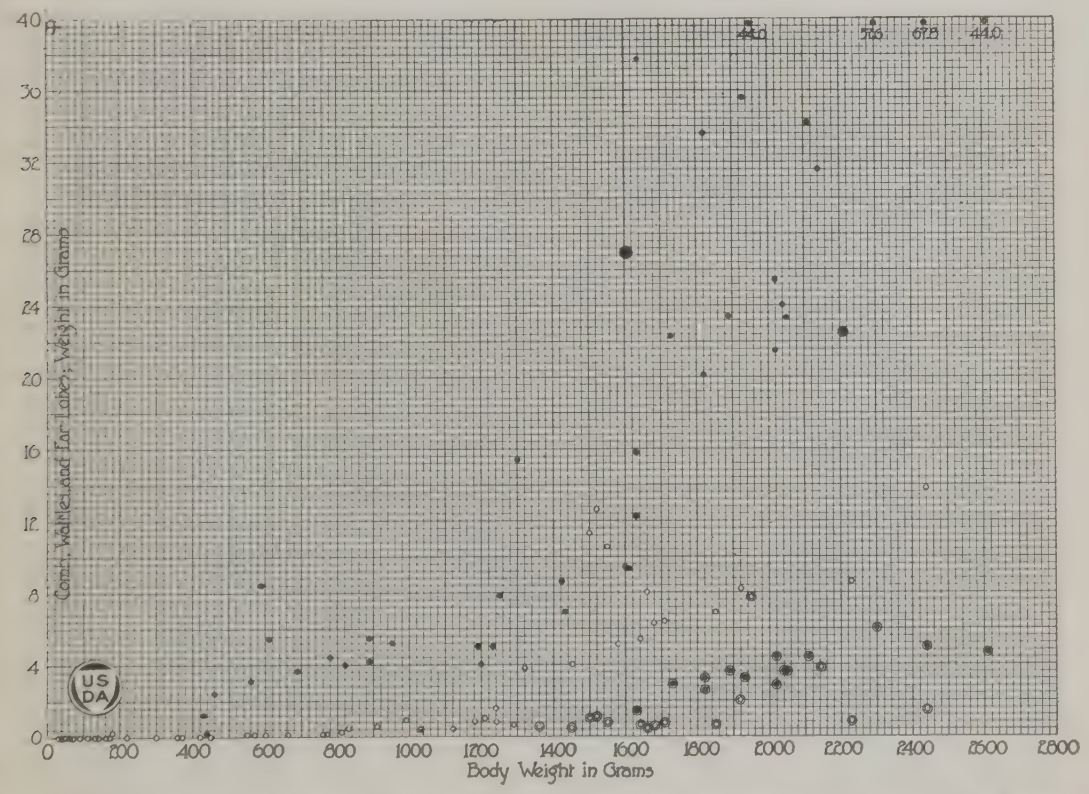

FIG. 10.—Individual weights of the combs and the two wattles (combined), plotted on gross body weight. Ear lobes are shown for the older chickens only. The encircled dots represent the ear lobes of the male; the double circles, weight of the ear lobes of the females. No curve was drawn for this chart since the data are too irregular

COMB AND WATTLES.—Figure 10 shows the absolute weights of the comb and wattles combined, and the weights of the two ear lobes (combined) for the older chickens. All are

plotted against the gross body weight. No formula or curve was made for this chart because the individual cases shown on the chart are too variable. In the very young chicks the wattles were so small that they could not be separated from the integument over the mandible. As soon as they could be distinguished they were removed and weighed with the comb. When plotted against age or body weight, the comb and wattles of the male birds appear more irregular in their growth than those of the females. The ear lobes were not weighed in all cases and hence are not shown for all the chickens. Their increase in weight appears to be more regular than that of the comb and wattles.

The muscles and integument form nearly straight lines when thus plotted, as shown in Figures 8 and 11. The musculature was also plotted on age, and the resulting curve resembled the curve of gross body weight (fig. 1). Figure 11 shows possibly a very slight sex difference in the heavier chickens, but only the one curve and formula are given. The percentage weights of the muscles show an increase from about 21 or 22 per cent of the net body weight at hatching to a little over 50 per cent for some of the larger chickens. The musculature of the three older cockerels averages about 50 per cent while that of the three hens averages only about 43 per cent. There is a similar difference in the older chickens in this series, the muscles of the males forming a larger percentage of the net body weight than do those of the females. It is popularly supposed that there is relatively more "meat" (muscle) in a hen than in a rooster, but the present data show that this is incorrect.

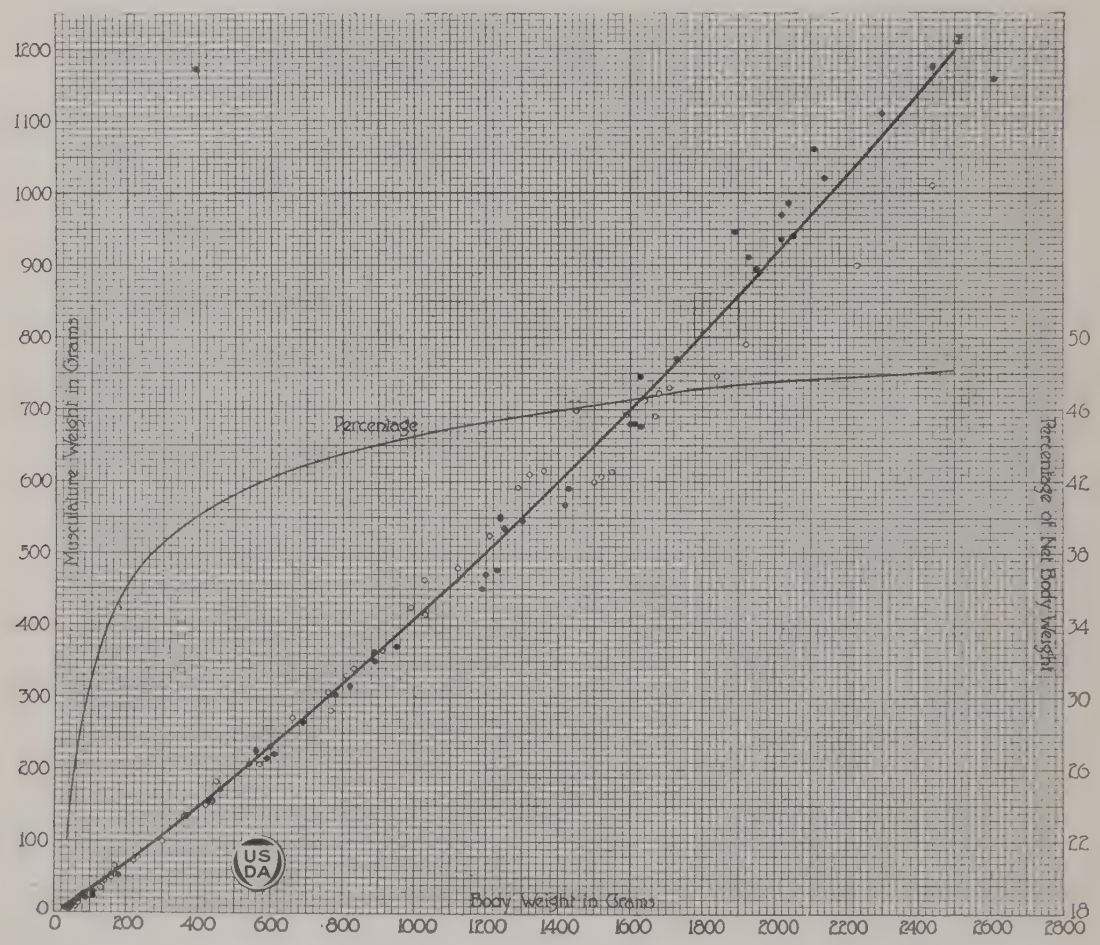

FIG. 11.—Weight of the muscles in gm. as the heavier line. The lighter and more sharply curved line represents the percentage weights of the musculature. Both are plotted on gross body weight

MUSCULAR SYSTEM

The absolute weight of the skeletal musculature and also its percentage weight, plotted against gross body weight, are shown in Figure 11. The formula for this chart is:

$$Y=(0.01X)^{1.8}+0.35X-5$$

from 50–2,600 gm. gross body weight.

Y represents the weight of the musculature in grams and X represents the gross body weight in grams.

The musculature in the chicken at first increases in weight more rapidly than the rest of the body and later forms about 50 per cent of the net body weight, with no marked sex

difference. There is no postnatal decrease in percentage weight of muscle, as described by Jackson and Lowrey (*9*) for the rat.

The relative weight of the muscular system seems to vary greatly in different species. According to Welcker and Brandt (*28*) it varies from about 19 per cent in the tortoise to nearly 59 per cent in the perch. Their average for the domestic fowl was 54.5 per cent. Jackson and Lowrey (*9*) have pointed out that the percentage weight of the muscles does not vary in proportion to the size of the animal. There seems to be some evidence to indicate that within a group or phylum of animals, the activity, or the ability to perform powerful or rapid movements, is correlated with the relative weight of the musculature in the animal.

Y represents the weight of the moist ligamentous skeleton in grams and X represents the gross body weight in grams.

This curve shows no sex difference until a body weight of 900 gm. is reached. Unfortunately, there are no male skeletons at body weights between 950 and 1,190 gm., but there is probably no marked change, since the curve for the males continues in a straight line up to 1,200 gm gross body weight.

At about 900 gm. of gross body weight there comes a marked change in the curve for the female skeletons; it continues to increase but not nearly so rapidly as before this time. The curve for the male skeleton continues at the same rate until 1,200 gm. in body weight is reached. From this point on, the

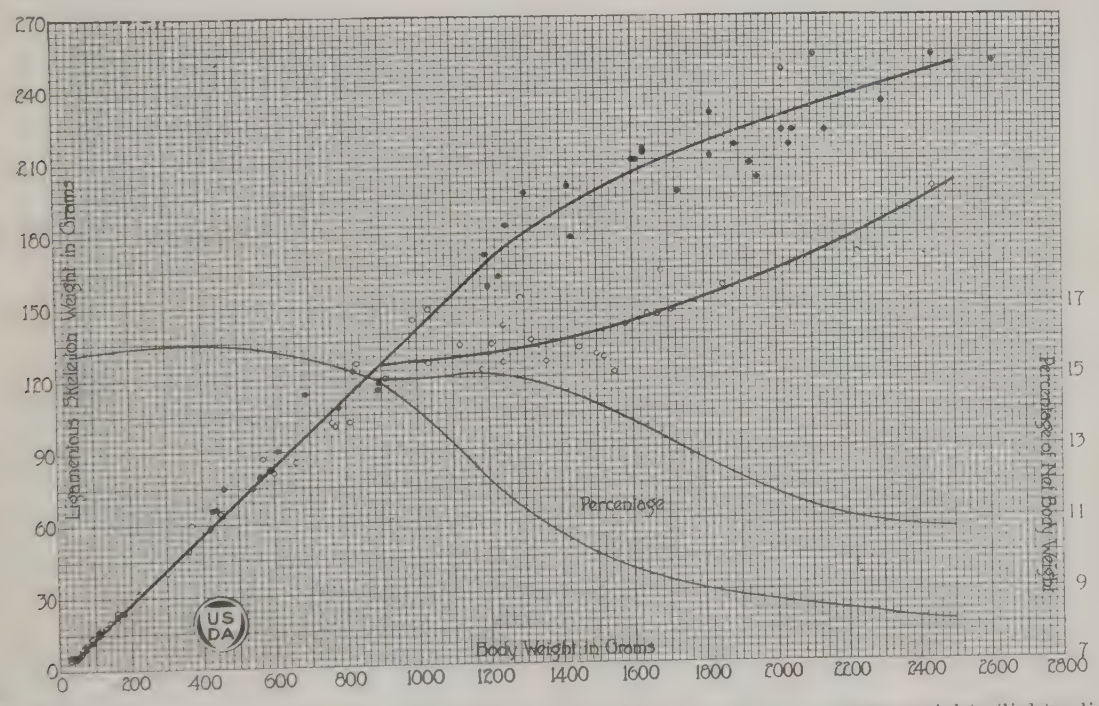

FIG. 12.—The curves represent the absolute weights (heavier line) and the percentage weights (lighter line) of the moist ligamentous skeleton, plotted on gross body weight

SKELETAL SYSTEM

Figure 12 shows the absolute and the percentage weights of the moist ligamentous skeleton, plotted against gross body weight. The formulas for the males are as follows:

$$Y = 0.14X$$

up to 1,200 gm. gross body weight,

$$Y = (X - 1100)^{0.7} - 0.04X + 190.88$$

from 1,200–2,500 gm. gross body weight.

For the females the formulas are:

$$Y = 0.14X$$

up to 900 gm. gross body weight,

$$Y = (0.01X)^{1.7} - 0.075X + 151.64$$

from 900–2,400 gm. gross body weight.

male, too, shows a relatively slower skeletal growth.

The cases above 2,200 grams in gross body weight shown on the chart are the six older chickens. It is seen that these fit into the curve for the younger chickens, indicating that there is no radical change in the skeletal weight after about 300 days. When the weights of the moist ligamentous skeleton are plotted against age there is even a more evident sex difference, the curves of male and female skeleton separating at about 120 days of age. This appearance of a sex difference in weight of the skeleton corresponds rather closely to the beginning of a sex difference in the growth of the linear measurements of the charts shown in Figures 3 to 5.

The relative weight of the skeleton shows at first a very slight increase attaining a maximum average of about 16 per cent and then decreasing to about 11 per cent for the males and about 8 per cent for the females. The greater percentage weight of the moist ligamentous skeleton in the cockerels can not be accounted for entirely by the smaller proportion of fat or of reproductive tract in the male. It must be due to other differences in the structure of the birds. The cockerel is characteristically longer-legged than the pullet. Moreover, the shape of the body is different, as mentioned previously, the cockerels having a larger anterior part of the body, which contains more bony skeleton, while the pullets have a relatively larger posterior part of the body which is not so well supplied with skeleton.

left side. In no specimen were more than eight pairs of ribs found.

DIGESTIVE SYSTEM

DIGESTIVE TUBE.—The chart in Figure 13 shows the growth in weight of the entire digestive tube, without contents, and the percentage weight, plotted against the gross body weight. The formulas are:

$$Y = X^{0.62} + 0.0053X - 4.91$$

from 30–1,400 gm. gross body weight,

$$Y = 0.013(X - 1{,}400) + 91.74$$

from 1,400–2,500 gm. gross body weight.

Y represents the weight in grams of the entire digestive tube, and X, the gross body weight in grams.

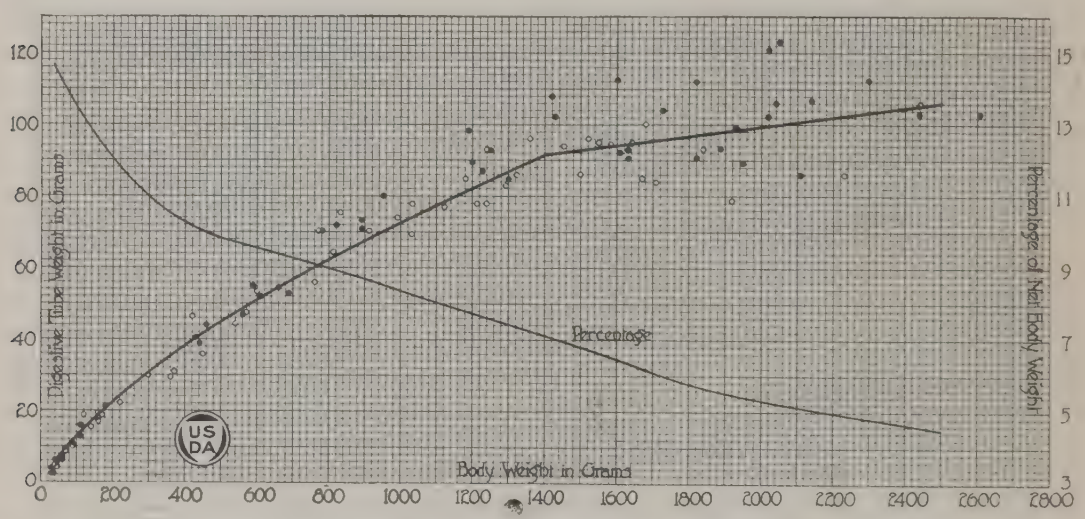

FIG. 13.—Changes in the absolute and relative or percentage weights of the digestive tube (without contents), plotted on gross body weight

The sex difference in the ligamentous skeleton of the chicken is also apparent in the relative (percentage) weight above 900 gm. in body weight. The figures given by Jackson and Lowrey (*9*) for the relative skeletal weight in the rat are slightly higher, and show no sex difference. Welcker and Brandt (*28*) give 11.69 per cent as the average for the two male chickens.

In making the autopsies on the 100 chickens, 11 (7 males, 4 females) were found with extra ribs. These varied from short pieces of bone imbedded in the body wall and not connected at either end with other skeletal elements to complete and normally articulated ribs. These extra ribs were found on one or both sides of the body. In seven males there were seven with extra right ribs and four with extra ribs on the left side. The four pullets with extra ribs had four with extra right ribs and three with extra ribs on the

This curve shows a more rapid rise at first followed by a second phase of slower growth. There is no apparent sex difference. The weights of the digestive tube when plotted on age form a curve strikingly like that in Figure 13, except that the first part of the curve is concave on its upper side and there is a slight difference in the curves for the males and females. In this case (as also for other organs) the curve for the male digestive tube is higher, corresponding to the greater body weight of the males. The curve of the relative or percentage weights shows a continuous decrease from about 15 per cent to about 4.5 per cent. During the first few days there is an increase in the percentage weight, but this is not indicated in the chart. The percentage weights of the empty tube in the nine chicks ranging in age from day of hatching to 8 days are as follows, in order of age; 8, 8.2, 13.6, 12.4, 14, 13.9,

18.5 (for the sixth day, the highest of all), 15 and 14.

Welcker and Brandt (*28*) give 5.02 per cent as the average for the canal in their two male chickens (adult). This is very close to that of the older Minnesota cockerels and but slightly higher than that of the hens. Jackson (*8*) gives about the same percentage values for the adult rat (esophagus not included), but finds that it increases from an average of about 2.4 per cent in the newborn to a maximum of about 8 per cent at six weeks of age. Thus the maximum relative weight of the digestive tube is attained much earlier in the chick and at this time it forms twice the relative weight of the tube in the rat. The greater weight of the tract in the chick at the beginning may be correlated with the difference in diet. In addition the chicken has the heavy-walled gizzard which frequently equals the weight of the intestines, crop, and esophagus together.

The cases appear more irregularly scattered than in the charts of the other parts of the digestive tube, for perhaps three reasons: (1) The scale of the ordinates is larger; (2) the separation of the stomach from the rest of the digestive tube was rather difficult, for there is no sharp line of separation between it and the esophagus; (3) the proventriculus may vary in size more than the other parts. The curves of both absolute and relative weights are very similar to the curves for the entire digestive tube (fig. 13).

GIZZARD.—Figure 15 shows the absolute weights of the gizzard, separately plotted against gross body weight. The lighter line shows the percentage weights. The formulas are:

$$Y = X^{0.48} + 0.01X - 5.12$$

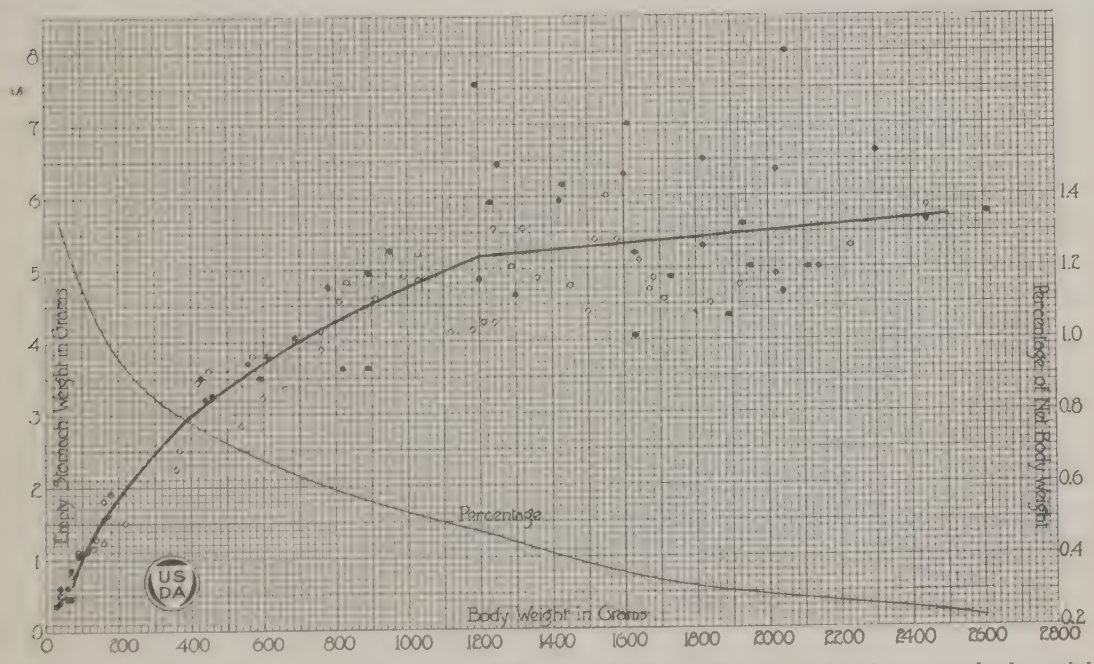

FIG. 14.—Growth of the stomach or proventriculus (without contents), plotted on gross body weight

STOMACH.—Figure 14 shows the growth in the absolute and relative weight of the stomach or proventriculus, plotted against gross body weight. The formulas are:

$$Y = X^{0.3} - 0.0002X - 2.96$$

from 80–1,200 gm. gross body weight,

$$Y = 0.00042\ (X - 1,200) + 5.19$$

from 1,200–2,500 gm. gross body weight.

Y represents the weight in grams of the stomach and X represents the gross body weight in grams.

from 80–1,500 gm. gross body weight,

$$Y = 0.0062\ (X - 1,500) + 43.33$$

from 1,500–2,500 gm. gross body weight.

Y represents the weight of the gizzard in grams and X, the gross body weight in grams.

The relations are discussed in the following paragraph.

INTESTINES.—Figure 16 shows the absolute and relative weights of the intestines (also including crop and esophagus) plotted against the gross body weight. The formulas are:

$$Y = (X + 100)^{0.57} - 0.002X - 14.57$$

from 30–1,200 gm. gross body weight,

$$Y = 0.0057\ (X - 1,200) + 42.59$$

from 1,200–2,500 gm. gross body weight.

Y represents the weight in grams of the intestines and *X* the gross body weight in grams.

The large paired caeca are also included with the weights of the intestine. A comparison of the charts in Figures 15 and 16 shows that, while the intestines and gizzard grow at very nearly the same rate, yet the gizzard lags behind the intestines throughout the middle part of the curve. This would indicate that the intestines complete their growth a little earlier than the gizzard.

The three regions into which the digestive tube was divided grow apparently at about the same rate. A comparison of the charts (figs. 14, 15, 16) will show a marked similarity. The relative weight of the intestines compared with the net body weight shows a short rise and then a decreasing rate of growth until in the adults the intestine forms about 2 per cent of the net body weight. This short initial increase in percentage weight is found also in the pancreas, liver, and kidney, all organs concerned with nutrition and excretion, which up to this time have been carried on by the yolk sac and allantois. It looks as though these organs made a rapid growth to meet the demands of the organism as soon as it had to provide for itself. The proventriculus or glandular stomach does not show this early rapid growth.

CONTENTS OF DIGESTIVE TUBE.—In connection with the digestive tube may be mentioned the contents found therein. The stomach never contained much food and usually showed none, while all the other parts of the canal (from crop onward) were generally more or less filled. In the stomach or proventriculus was always found a characteristic whitish mucus lining the walls, which were thrown into longitudinal folds.

The use of gross or net body weights for abscissae and for calculation of percentage weights raised the question of the amount of tare or contents of the digestive tube. For abscissae it seemed best to use the gross weights because these are more easily determined and more generally useful for reference; but for calculating percentage weights, the net body weight was used. It was thought that a corrective factor could make possible the conversion of gross into net weight, or vice versa. The absolute and the percentage weights of the tare were therefore plotted on age, but there appeared a surprising variability in both cases. As might be expected the increase in contents is very rapid at first.

Just after hatching and for the succeeding three days the absolute weights are: 0.7, 1.4, 3.7, and 6 gm. Until a little after the one hundredth day the variation in absolute weight of the tare is not great, but after that day the range is from 30.9 gm. to 160.9 gm. The relative weights for the first six days following the date of hatching are: 2.3, 3.95, 12.1, 15.8, 14.3, 18.2, and 19.3 per cent, reaching the maximum found in any specimen. While the cases are very irregularly arranged, their average slowly falls after the sixth day to about 4.5 per cent of tare for the adults. The use of this number as a means of changing from gross to net body weight would give only approximate results, however, for the variation is very great.

A part of the tare in the fowl is the gravel in the gizzard. In a majority of the older chickens, some grit or small stones appeared in the large intestine. Why they should be more

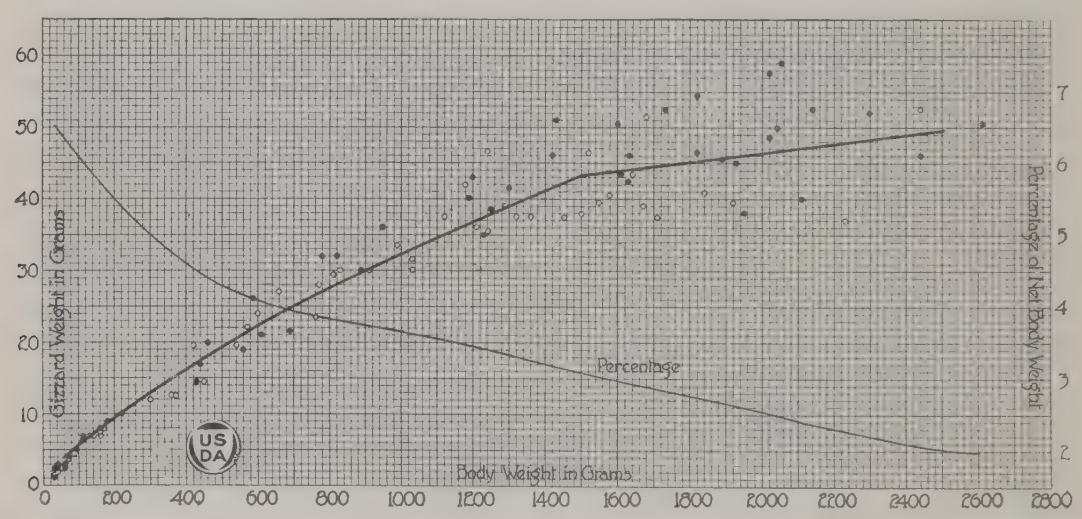

FIG. 15.—Absolute and relative or percentage weights of the empty gizzard. The heavier line represents the weight in grams. The lighter line shows the percentage of the net body weight

abundant in this part of the intestine is not easy to say. The presence of grit in the posterior part of the intestine was not observed in the younger chicks. Fine sand was found in quantity in the gizzard of the one-day-old chick, or before there was any food present.

Jackson and Lowrey (*9*) find that the intestinal contents for the albino rat during postnatal life do not average more than 5 per cent of the body, excepting at 6 weeks, when the average was about 8 per cent.

YOLK SAC.—As is well known, the yolk sac is inclosed by the abdominal wall just before hatching, and the resulting scar can readily be seen on the young chick just after hatching. Aristotle long ago observed the yolk sac 10 days after hatching; and William Harvey found it in a chick 13 days old. H. Virchow (*27*) found the yolk sac weighing an average of 5.34 gm. just after hatching, but reduced to about 0.05 gm. on the sixth day. Kaupp (*11*) reported an average weight of 8.5 gm. for the yolk sac in 10 newly hatched, single-comb White Leghorn chicks. At 43 hours he found it reduced to 5 gm., and still present at 120 hours, although the weight is not given.

The findings concerning postnatal persistence of the yolk sac are as follows in the present series: The yolk sac was found in every chick, with one exception (thirty-first day), up to and including the thirty-sixth day. From 38 to 108 days, inclusive, it was present 15 times and not found in 12 chicks. After the age of 108 days, the yolk sac was found in 8 chickens (4 males, 4 females), the older being a cockerel 237 days old. It was exceedingly variable in weight. For example, the first yolk sac weighed 5.05 gm.; at one day, 7.56 gm. (the heaviest found). In the chick at 2 days it weighed but 1.4 gm. and at 12 days, 6.01 gm.

TABLE I.—*Data for the oldest six cases in which the yolk sac was found*

Sex	Age in days	Gross body weight	Yolk sac weight	Percentage of net body weight
		Gm.	*Gm.*	
Male	151	1,633.2	0.079	0.0050
Female	167	1,324.7	.058	.0046
Male	172	1,820.9	.822	.0480
Female	181	1,363.8	(*a*)	(*a*)
Do	202	1,707.3	.033	.0020
Male	237	2,023.5	.858	.0459

a Yolk sac was 2 mm. in diameter, but was not weighed.

The yolk sac was found in one of two conditions. Either it was soft in consistency and more or less imbedded in the abdominal wall (as was the last one), or it seemed to have broken free from its attachments and formed a hard spherical structure, filled with a more or less chalky mass, in various positions in the abdominal cavity and with very slight or no attachments.

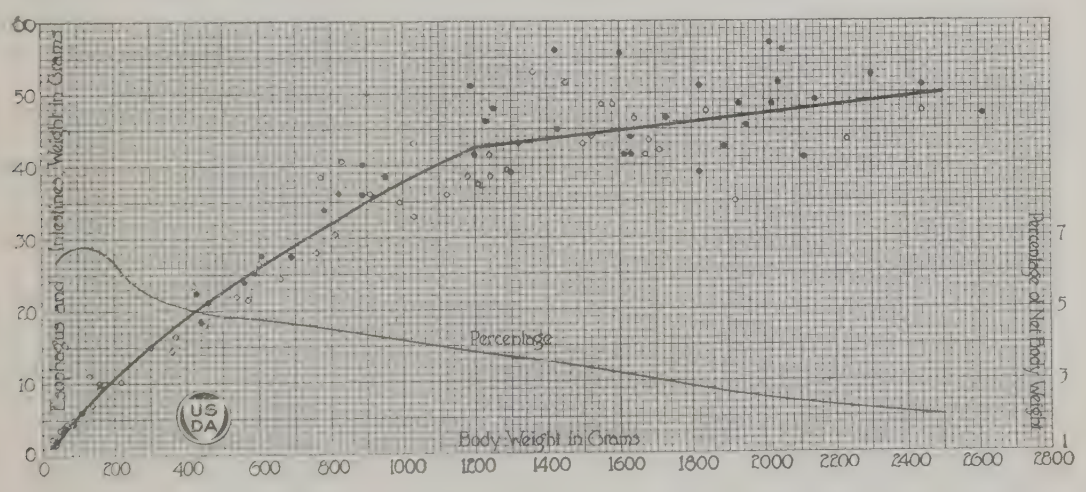

FIG. 16.—Weight of the esophagus, crop, and intestines (all without contents) plotted on gross body weight as the heavier line. Percentage weights plotted as the lighter line

Meckel's diverticulum, or the primitive attachment of the yolk stalk, persists constantly as a small tubular outgrowth from the free side of the intestine. It was 6 mm. in length in the 230-day chick, which was about the average. There seems to be a tendency for this to shorten very slightly with age, but even in the hens 2 years old it was present as a small diverticulum several millimeters in length and always with a lumen open widely into the intestine.

LIVER.—The growth in weight of the liver and gall bladder (fig. 17) follows the same general type of growth curve as the other parts of the digestive tract. There is no significant sex difference. The formulas are:

$$Y = X^{0.44} + 0.017X - 5.29$$

from 70–1,500 gm. gross body weight,

$$Y = 0.00715\ (X - 1,500) + 45.18$$

from 1,500–2,400 gm. gross body weight.

Y represents the weight of the liver in grams and *X* represents the gross body weight in grams.

The percentage weights of the liver form a curve also resembling the percentage curves for the other parts of the digestive system, although the changes are not so marked. There is a short period of initial rise from 3.1 per cent at hatching to a maximum of 6.2 per cent at 7 days, followed by a slow decline to about 2.5 per cent. There seems to be a tendency for the liver in the females to run higher (possibly 1 per cent higher) than in the males. This is reversed for the six older chickens, for in these the percentage weights of the livers in the males average higher than in the females. After the chickens become mature, they become very fat, especially the females. In some of these older fat chickens the liver had a yellowish color, and these are the heavier specimens in Figure 17. For example, the last three cases (not included in the curve) were adults which were very fat and had the yellowish-colored livers. The female at 1,640 gm. had a yellow liver, also the females at 1,520 and 1,550 gm. The male at 1,600 and the female at 1,840 gm. also have heavy livers, but these livers were not specifically observed to be yellowish in color. (They may have been so, with no mention of the fact made on the record cards.) The storage of additional fat, glycogen, etc., in the heavy livers is a possible explanation for the marked increase in the weight of the liver in the last three cases on the chart and for at least some of the heavier cases recorded for chickens under 2,400 gm. In the very young chicks the liver had a yellowish color, but on the fourth day the liver was pinkish, and from then on rapidly changed to the characteristic dark red in the older birds.

Zaitschek (*31*) reports no appreciable change in the percentage values of the livers in two chickens which he fattened for 55 days. At the end of the period they had gained 71.9 and 133.8 per cent of the initial gross body weight and yet their livers formed, respectively, 2.3 and 3 per cent of the live weight. The range in percentage weights of the liver in the 131 specimens reported by him was from 1.4 to 4.7 per cent.

Welcker and Brandt (*28*) give 1.88 per cent as the average percentage weight for the liver in two male fowls, which is lighter than that found in the present series. They record a variation from 1.68 to 4.74 per cent for the various other species of birds. The liver of the chicken is lighter than that of the rat as given by Jackson (*8*). He finds it forming 4.7 per cent at birth, increasing to nearly 8 per cent at 3 weeks, later decreasing to about 4.5 per cent in the adult.

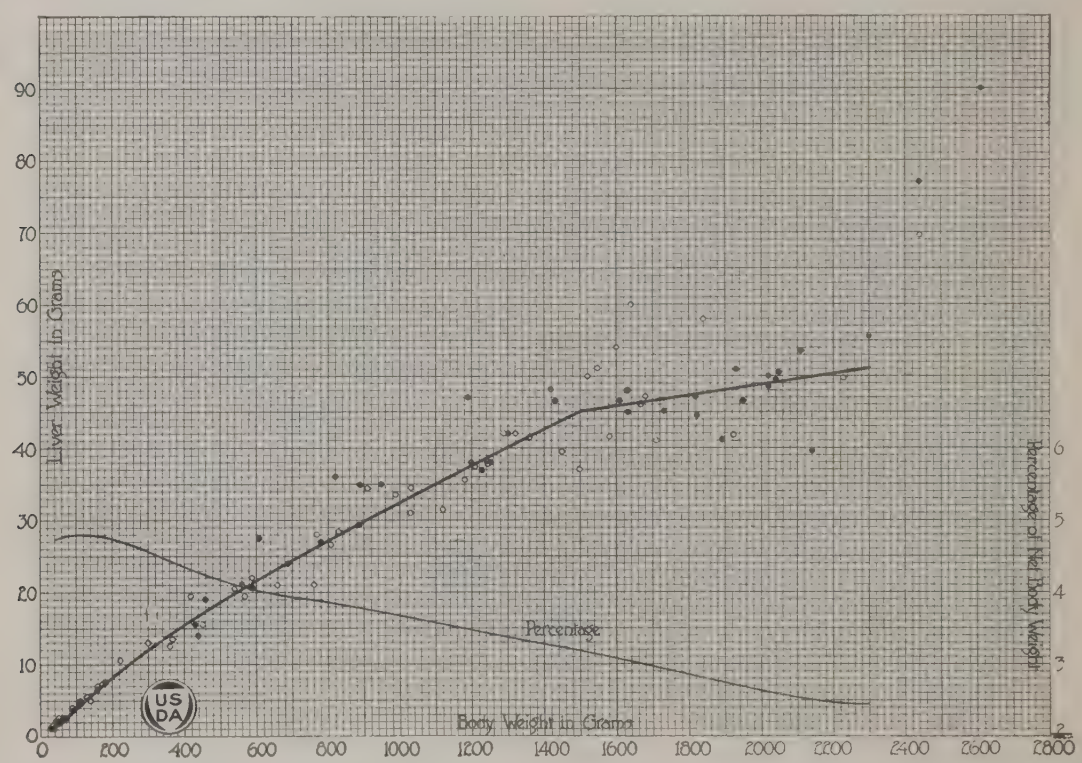

FIG. 17.—The percentage weight (lighter line) and the weight in grams (heavier line) of the liver. The three cases above 2,400 gm. gross body weight are shown on the chart though not included in the computation of the formulas

PANCREAS.—Figure 18 shows the increase in absolute and percentage weights of the pancreas plotted against gross body weight. The formulas are:

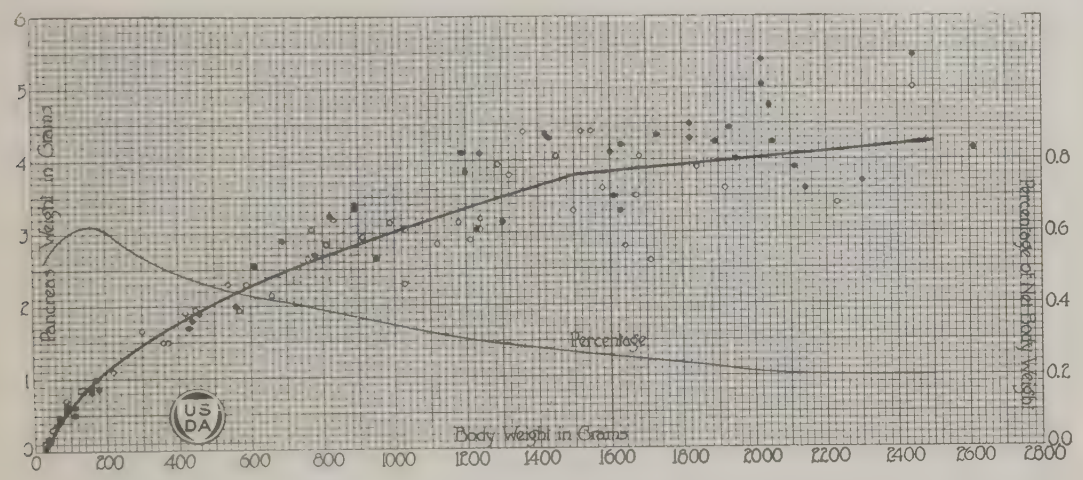

FIG. 18.—Weight in grams of the pancreas, shown by the heavier line with the individual cases. The lighter line represents the percentages of the net body weight

$$Y = X^{0.22} + 0.0007X - 2.23$$

from 40–1,500 gm. gross body weight,

$$Y = 0.00042\ (X - 1{,}500) + 3.82$$

from 1,500–2,500 gm. gross body weight.

plotted against age, except for an initial flattening of the first part of the curve. There seems to be a good deal of variation in the distribution of the cases, especially toward the upper end of the curve. This may be due in part, although not entirely, to the greater difficulty in removing the pancreas free from all fat, mesenteries, etc.

In general, by way of summary, it may be said that the parts of the digestive tract do not show any marked differences in their growth rates (figs. 13 to 17). The curve for the weight of the liver seems to differ slightly, increasing a little more slowly and uniformly throughout the entire range.

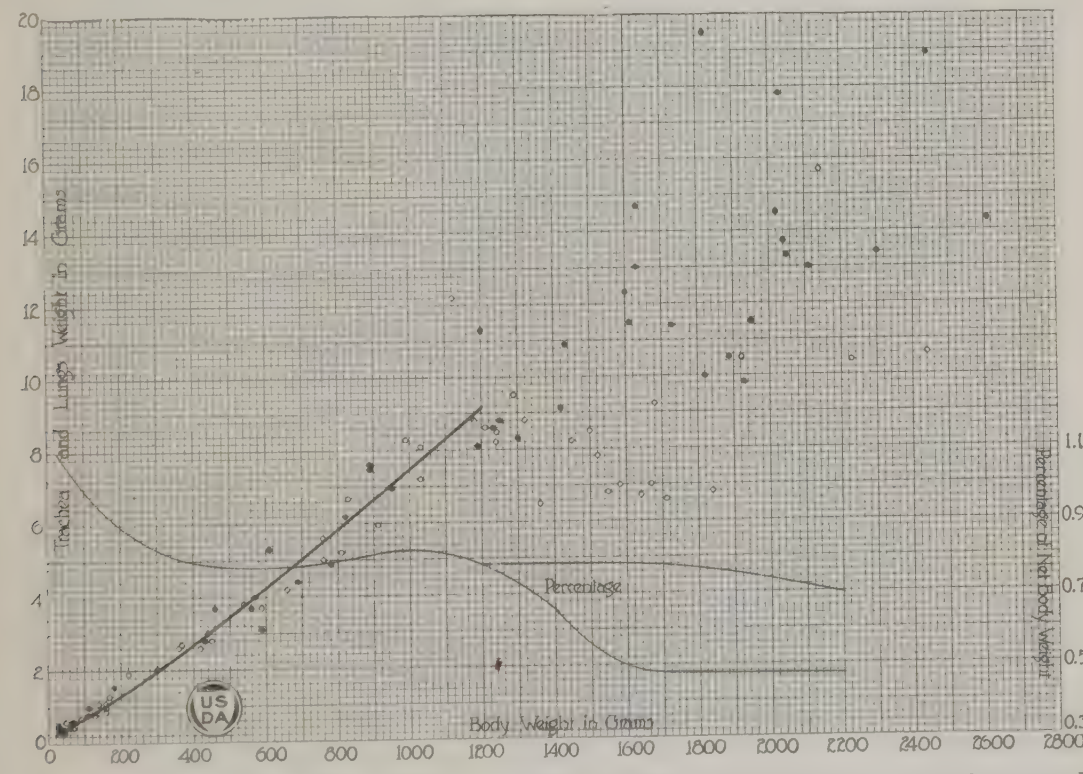

FIG. 19.—Absolute and percentage weights of the trachea and lungs (combined), plotted on gross body weight

Y represents the weight of the pancreas in grams and X the gross body weight also in grams.

This chart likewise resembles the other charts for the digestive system. Here also there is no marked difference in the curve when these weights are

RESPIRATORY SYSTEM (TRACHEA AND LUNGS)

The lungs and trachea were removed and weighed together. Their growth in gross and relative weight is shown in Figure 19. The formula is:

$$Y=(0.01X+1)^{1.1}-0.0056X-0.88$$

from 30–1,200 gm. gross body weight.

Y represents the weight of the liver in grams and X the gross body weight, also in grams. Up to 1,200 gm. in gross body weight, the cases fall fairly well in line; but above this weight there is a great deal of variation and consequently the mathematical curve is carried only this far. The same condition is seen when the weights are plotted on age. The cases are regularly arranged up to about 120 days, after which they are more irregular, especially in the males. When the blood was allowed to drain from the body as previously described a marked difference in the amount was noticed. In some specimens the blood coagulated much sooner and thus a larger amount remained in the chicken. Thompson and Carr (*26*) find a marked variation in the time of coagulation of the blood in the domestic fowl (Plymouth Rocks). The lungs are very vascular and this difference in the amount of blood may account for some of the variability of the lungs as well as some of the other vascular organs.

There is apparently a sex difference in the respiratory tract in the chickens above 1,200 gm. in body weight. This sex difference is also shown in the curve of the relative growth. The relative weights of the two sexes are shown by separate curves from 1,200–2,200 gm. gross body weight. There is a great deal of variation in the percentage values, and the curves shown in Figure 19 are merely the inspection curves drawn through the double-weighted medians. The relative weights for the individual chickens are not shown. Donaldson (*3*) shows no sex difference for the lungs in the rat, and no sex difference is found in the human species. It hardly seems probable that the crowing of the cockerel would make this difference, but this seems to be the only explanation for the apparent sex difference.

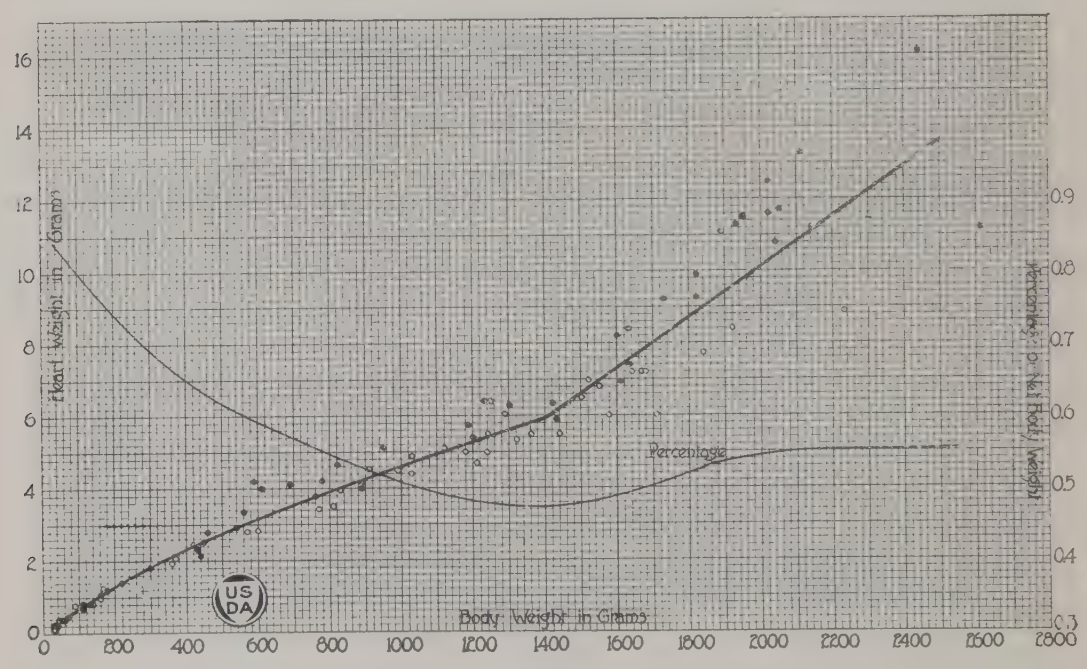

FIG. 20.—Growth of the heart ploted on gross body weight. The heavy line shows absolute weight. The lightert line shows relative or percentage weights

CIRCULATORY SYSTEM (HEART)

The weights of the heart in grams and the percentage weight plotted against the gross body weight are shown in Figure 20. The formulas are:

$$Y=X^{0.2}+0.0027X-2.08$$

from 50–1,400 gm. gross body weight,

$$Y=0.007(X-1,400)+5.96$$

from 1,400–2,500 gm. gross body weight.

Y represents the weight of the heart in grams and X the gross body weight in grams.

Here also as in the case of the lungs, there is an apparent tendency to a sex difference toward the end of the curve.

It is not, however, so evident for the heart as for the lungs. This chart shows that the heart increases a little more slowly during the first and middle portions of the curve but continues to increase in the heavier chickens. After 1,400 gm. in gross body weight, the increase in the heart weight is marked, while in the digestive system, for example, there is a much slower rate of increase after a body weight of 1,600 gm. is reached. This is in accord with the condition found in man, namely, that the heart increases slowly in weight after maturity.

The percentage weights of the heart show a very brief period of increase from 0.51 per cent at hatching to a maximum of 1 per cent on the fourth day. The curve of relative growth does not show this very brief period of initial increase but steadily decreases from about 0.85 per cent to 0.47 per cent at 1,400 gm. gross body weight. It then rises to a value of 0.55 per cent at 2,100 gm. gross body weight and remains nearly at this level to the end. In the individual percentage values (not shown on chart) there seems to be an indication of a relatively heavier heart in the male after about 1,200 gm. of gross body weight.

There is no apparent explanation for the relatively heavier heart in the cockerel any more than there is for the lungs. Possibly it may be associated with the increased activity of the cockerel, for as Joseph (*10*) has suggested, the activity of an animal is correlated with the size of the heart.

Jackson (*8*) found that in the rat the heart forms 0.65 per cent at birth, with a slight postnatal increase, gradually decreasing to about 0.4 per cent in the adult.

DUCTLESS GLANDS

THYROID GLAND.—The thyroid gland in the chicken is a rather small oval gland located on each side at the base of the neck, or at the bifurcation of the common carotid artery. It consists of a single lobe on each side. Figure 21 shows the absolute and the percentage weights of the gland plotted against gross body weight. The formula is:

$$Y = 0.01[0.01(X+100)]^{1.23} - 0.000075X - 0.006$$

from 30–2,100 gm. gross body weight.

Y represents the weight of the thyroid in grams and X represents the gross body weight.

In the smaller chicks it shows a much slower growth than do most of the organs, except the sex glands and the secondary sexual structures, such as the comb and wattles. The female at 1,290 gm. in body weight had an unusually heavy thyroid, of 0.417 gm. This weight was not averaged in the series, being excluded as either an abnormality or an error. All three of the older cockerels had much heavier thy-

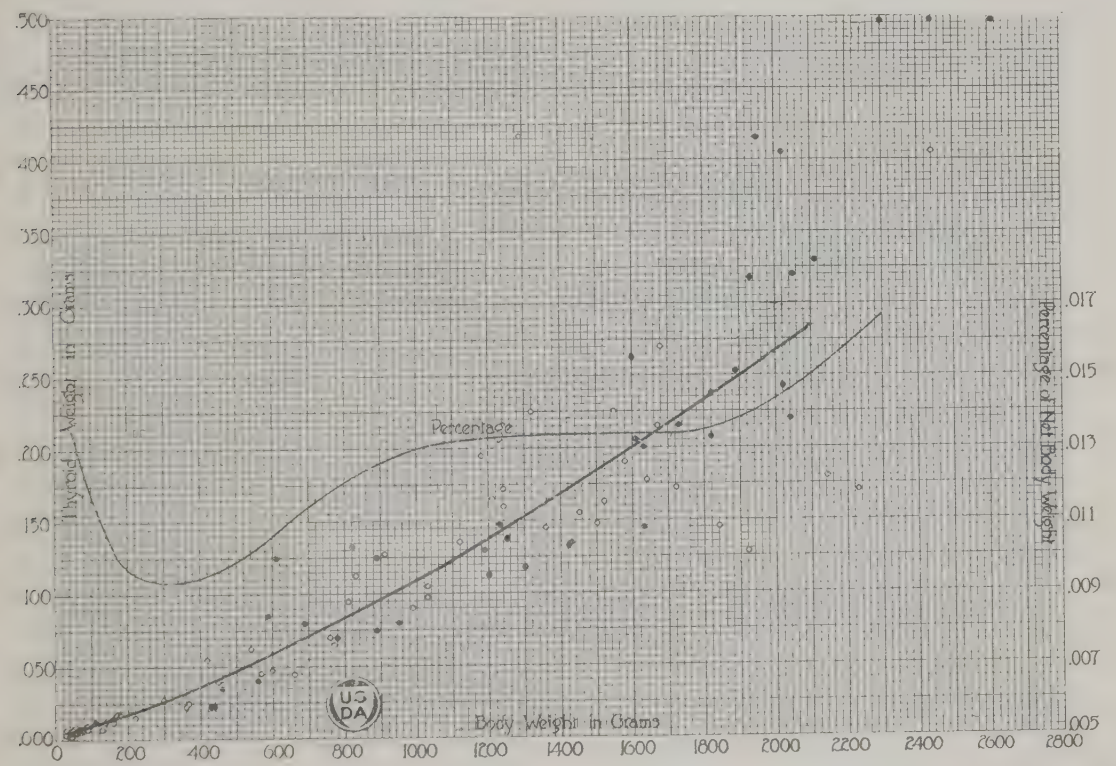

FIG. 21.—Absolute and relative weights of the thyroid gland plotted on gross body weight. All the cases above 2,200 gm. gross body weight or the adults, and the pullet at 1,290, and the hen at 1,920 gm. gross body weight, are shown in the chart though not included in the data used in determining the curve

roids. These and the thyroid glands from the three hens at 1,920, 2,230 and 2,440 gm. gross body weight were all excluded in the averages for the curve, although the individual cases are shown on the chart. There is apparently a sex difference in the weight of the thyroid in the six older chickens, but this may be due to a difference in diet. The three hens were taken from one of the pens of the regular laying stock, but the cockerels were from a lot of male birds isolated all winter, from which breeding cockerels had been selected. The larger birds, both male and female, from these pens were selected in order to extend the upper end of the curve based on gross body weight.

The percentage weights of the thyroid plotted on gross body weight are exceedingly variable. The lighter line represents the averages of the percentage weights of the thyroid but not the individual cases. There is no apparent sex difference until the upper end of the curve is reached. The curve shows an initial decrease from 0.013 per cent to nearly 0.009 per cent at 300 gm. gross body weight. It then rises to about its initial value at 1,100 gm. body weight, remaining at about this level up to 1,800 gm. of gross body weight, when it again rises. This last rise is caused by the larger percentage values in the adults. When the relative weights are plotted on age there is this same depressed portion of the curve falling between 40 to 60 days.

THYMUS.—The thymus of the chicken is located farther cephalad than the thyroid. It consists of a chain of lobes lying along each side of the neck, from the larynx down to the thyroid gland. The larger lobes, which persist longer when the gland begins to atrophy, are as a rule located at the base of the neck or nearer the thorax. The lobes are usually of a pinkish color, although some were much darker.

Figure 22 gives the absolute and relative weights of the thymus plotted against age in days. The formulas are:

$$Y=0.1\ [(0.1X)^{1.6}]$$

from 10–130 days,

$$Y=0.1\ [(0.1X)^{1.6}]-0.135\ (X-130)$$

from 130–260 days.

Y represents the weight of the thymus in grams and X represents the age in days.

The weights of the thymus plotted on gross body weight do not give nearly so regular a curve as that according to age, shown in Figure 22. The first or growth phase in each of the two curves is very similar, but the position of the older pullets, toward the center of the chart based on body weight, produces an irregular and more abrupt second or involution phase of the curve. Evidently the thymus involution in the chick depends on age rather than on body weight, as was found by Hatai (*6*) for the rat.

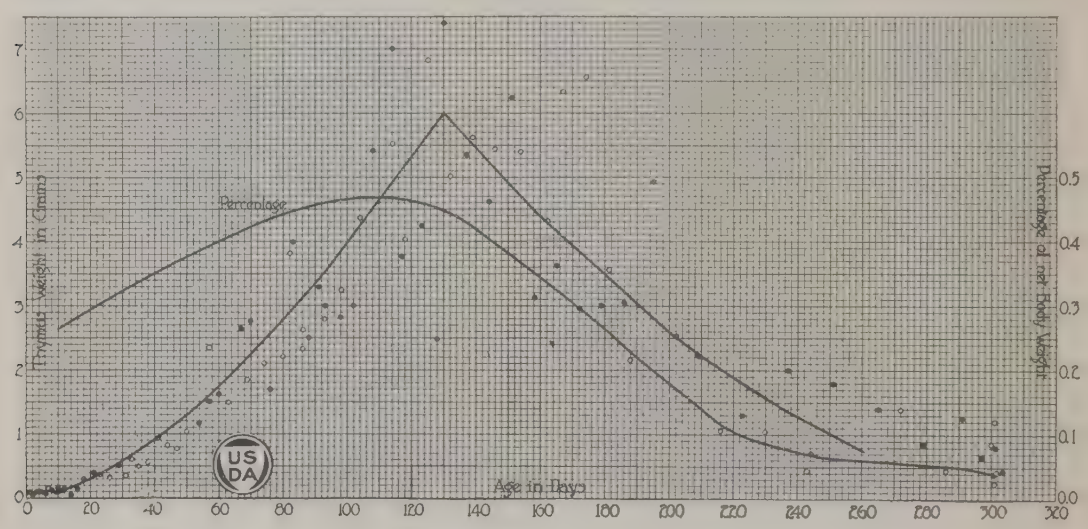

FIG. 22.—Absolute and relative or percentage weights of the thymus, plotted on age in days

The thymus of the chicken follows the usual course of development for the gland as found in other animals. It increases slightly more rapidly than the body up to 130 days, and then it decreases to nearly the same absolute weight as that of a chick about 40 days old. The relative weights in the older chickens are naturally much less. At first the thymus forms a little less than 0.3 per cent of the net body weight. This percentage rises until at 110 days it forms about 0.46 per cent; then there is a decrease until it reaches about 0.05 per cent for the older chickens of this series, and slightly less for the adult chickens.

The gross appearance of the involution changes indicate that the usual two methods of obliteration are found in the chicken, namely, a fatty or a fibrous involution. The lobes become smaller and later a decrease in the number of lobes is apparent. Throughout the entire period more or less fat was found surrounding the thymus and the lobes were enclosed by the fascia.

Jackson (*8*) found the following relative weights for the thymus in the male white rat: at birth it forms 0.15 per cent, which makes it slightly lighter than that of the chick; then it increases to a maximum of 0.38 per cent at 20 days, and again decreases to 0.02 per cent at one year. Since the rat matures more rapidly than the chicken, this earlier maximum in the growth of the thymus is to be expected.

SPLEEN.—Figure 23, which gives the absolute and relative weights of the spleen plotted against gross body weight, shows that the spleen is extremely variable in the chicken, as it has been found to be in other animals. The formulas are:

$$Y=X^{0.12}+0.00132X-1.74$$

from 100–1,200 gm. gross body weight,

$$Y=0.00039\ (X-1200)+2.18$$

from 1,200–2,600 gm. gross body weight.

Y represents the weight of the spleen in grams and X, the gross body weight in grams.

The relative weights show an initial increase followed by a slow decrease after 1,100 gm. in gross body weight.

Accessory spleens were noted in 4 specimens (2 males, 2 females), ranging in age from 54 days to 237 days, or from 426.6–2,023.5 gm. in gross body weight. They were found in the neck, at the anterior end of the crop, close to the hilum of the spleen, and in the 237-day female, near the pancreas on the opposite side from the normal spleen.

SUPRARENAL GLANDS.—Figure 24 shows the absolute and relative growth of the suprarenal glands plotted against gross body weight. The formula is:

$$Y=X^{0.012}+0.000077X-1.046$$

from 100–2,600 gm. gross body weight.

Y represents the weight of the suprarenal gland in grams and X, the gross body weight in grams.

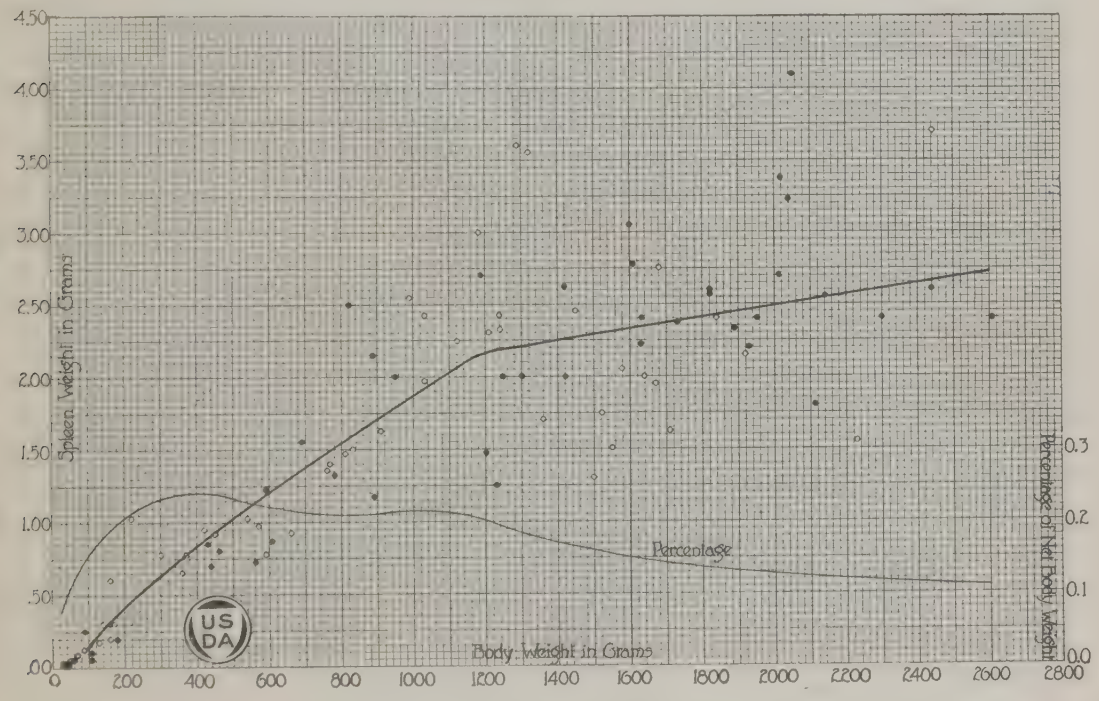

FIG. 23.—Absolute and relative or percentage weights of the spleen, plotted on gross body weight

Some of the irregularity in the upper part of this curve may be due to the difficulty in removing these glands. In the female the left suprarenal is lodged at the base of the ovarian ligament, and when the ovary is fully developed the ligament is so strong and tough that the removal of the left suprarenal gland without injuring it is difficult.

There is possibly a very slight increase in the percentage weights of the suprarenal after hatching, with a maximum value for one specimen of 0.0359 per cent on the fourth day. Then there is a decrease until the average for the adult is about 0.01 per cent. These values are slightly greater for the maximum and a little less for the adult than given by Jackson (*8*) for the male rat.

The suprarenals are considerably larger in the female rat. Unlike the rat, the chicken shows no apparent sex difference.

HYPOPHYSIS.—The hypophysis of the chicken seems to be quite variable in weight, as shown in Figure 25. The formulas are:

$$Y=0.001(X^{0.34}-0.001X-1.49)$$

from 100–1,600 gm. gross body weight,

$$Y=0.0000063(X-1600)+0.00919$$

from 1,600–2,600 gm. gross body weight.

Y represents the weight of the hypophysis in grams and X, the gross body weight in grams.

no appreciable sex difference when the weights are plotted against body weight. If there is any difference at all it is rather in favor of a heavier male hypophysis.

UROGENITAL SYSTEM

KIDNEYS.—The growth of the kidneys plotted against gross body weight (fig. 26) shows a marked initial rise followed by a gradual decrease in rate of growth. The formula is:

$$Y=(0.1X)^{0.6}-0.0041X-2.17$$

from 100–2,600 gm. gross body weight.

Y represents the weight of the kidneys in grams and X represents the gross body weight in grams.

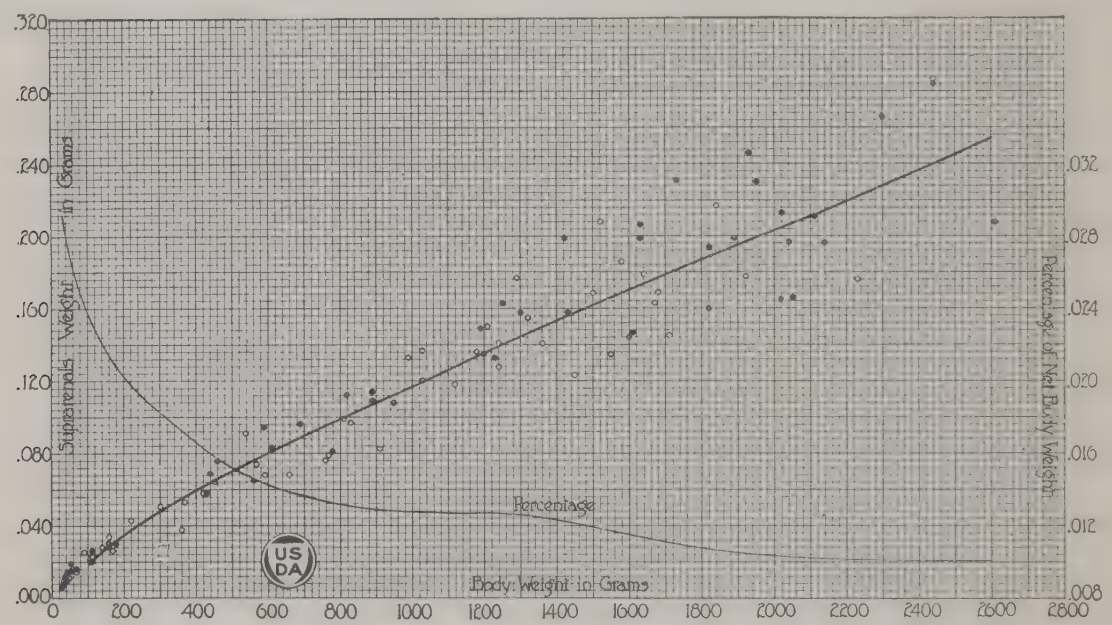

FIG. 24.—Relative and absolute weights of the suprarenal glands, plotted against gross body weight. There is no apparent sex difference

A similar irregularity in the arrangement of the cases is observed when age rather than gross body weight is used for the abscissae. The percentage weights are also variable at first (possibly because of difficulty in removal). After this first irregular period with an average of about 0.078 per cent of the net body weight, there is a rather precipitous fall in the percentage weights to about 0.0015 per cent followed by a slow decrease to about 0.0006 per cent, which is also the average percentage weight for the three older cockerels. The hypophyses of the three hens average about 0.0005 per cent.

In the rat there is a marked sex difference in the weight of the hypophysis, the female being the heavier according to Hatai (*5*) and Donaldson (*3*). The data on the chick show

No significant sex difference nor peculiarities in the weight of the kidneys appear in the older cockerels and pullets.

The percentage weights show a marked initial rise followed by the usual decrease, and a terminal irregular portion. At hatching the kidneys form 0.6 per cent of the body weight, but they rise rapidly until at 5 days they form 2 per cent, which is the highest case of the entire series. The average for the older birds would be about 0.7 per cent, with the adult cases a little less. The older pullets seem to have slightly heavier kidneys, relative to body weight, than do the cockerels of the same age.

Welcker and Brandt (*28*) give 0.59 per cent for the kidneys in two adult male chickens, which is about the same as the average percentage values for

the three older cockerels of the present investigation. Jackson (*8*) gives the following percentage weights for the kidneys of the male rat: At birth, 0.96 per cent; at seven days, 1.29 per cent; and a maximum of 1.44 per cent at twenty days; it then decreases to 0.95 per cent at one year.

OVARY AND OVIDUCT.—Figure 27 shows the increase in weight of the ovary (large dot) and oviduct (circle) plotted against age. The distribution of cases is very irregular but less so than when plotted against body weight. No attempt has been made to draw a curve or derive a formula for these organs, since both are very irregular in their growth and the number of cases is not sufficient to justify a curve. The tinues to be the heavier, with two individual exceptions: The pullet at 286 days and one of the 2-year-old hens.

The pullet at 188 days had a fully developed egg in the lower end of the oviduct and the following day an egg was laid by one of the other pullets of this group (Group 1). Between the pullet at 181 days and that at 188 days, in an interval of seven days, the oviduct shows an increase from 8.66 gm. to 82.75 gm., or about 8.6 times; while the ovary has increased from 2.108 gm. to 38.94 gm. or 17.5 times. In addition, the ovary and oviduct have produced the egg which was found in the oviduct of the pullet at 188 days.

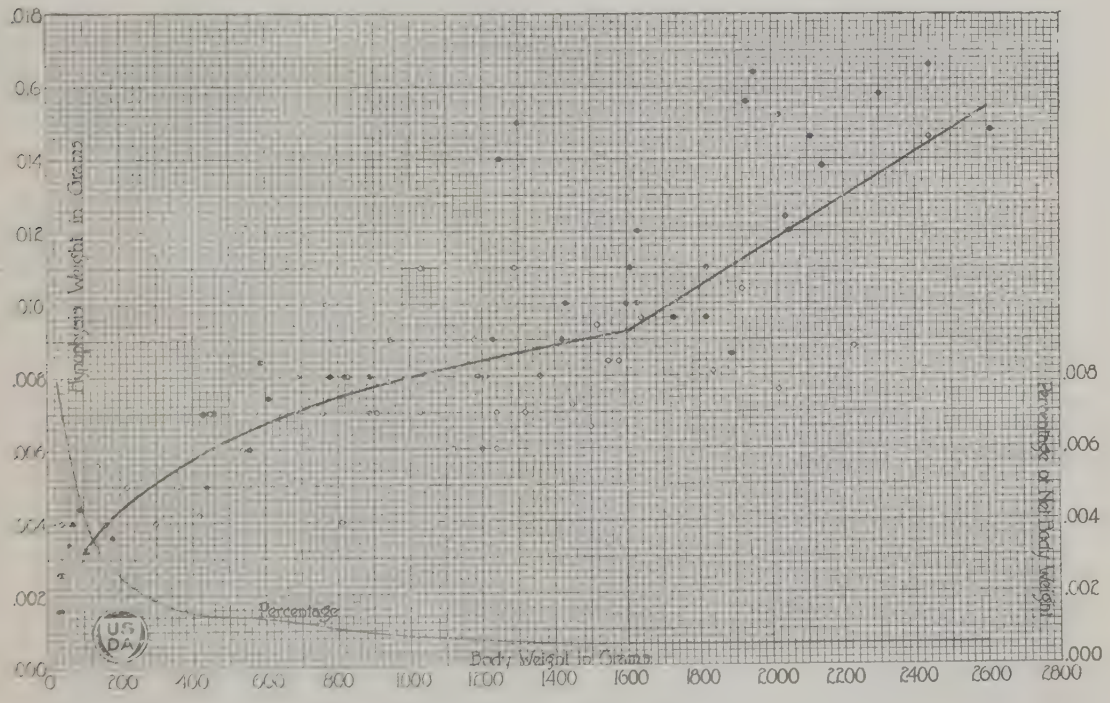

FIG. 25.—Absolute and relative weights of the hypophysis, plotted on gross body weight

weights are for the empty oviduct. One egg (never more) was sometimes found in the oviduct, but it was removed before weighing the egg tube.

Four phases of ovarian growth are recognized: (1) an initial period of very slow growth, followed by (2) a slight increase in growth rate; then (3) a period of very slow growth followed by (4) a second period of more rapid growth. There is a brief prepuberal period of rapid growth which lasts but about 30 days, or from about 160 to 190 days of age.

The oviduct was not weighed in the smaller chicks, but from about 80 days up to 160 days of age it was always a little lighter than the ovary. In the pullet at 162 days the positions on the chart were reversed, the oviduct becoming heavier. The oviduct con-

TABLE II.—*Growth in diameter of the largest ovum in the ovaries of the pullets of Series I*

Chick number	Age	Diameter of largest ovum
	Days	*Mm.*
I-31	162	4.5
I-33	167	3.0
I-35	174	7.0
I-37	181	9.0
I-39	188	(a)

a Mature egg in terminal part of oviduct.

The relative weights of ovary and oviduct undergo a correspondingly large increase. The oviduct increases

from 0.69 per cent to 5.62 per cent, or 7.1 times; and the ovary increases from 0.17 per cent to 2.65 per cent, or 14.5 times. No eggs were found in the oviducts of the pullets at 230 and 243 days. The pullet at 243 days is from Group 3, the other pullets above 188 days are all from Group 1.

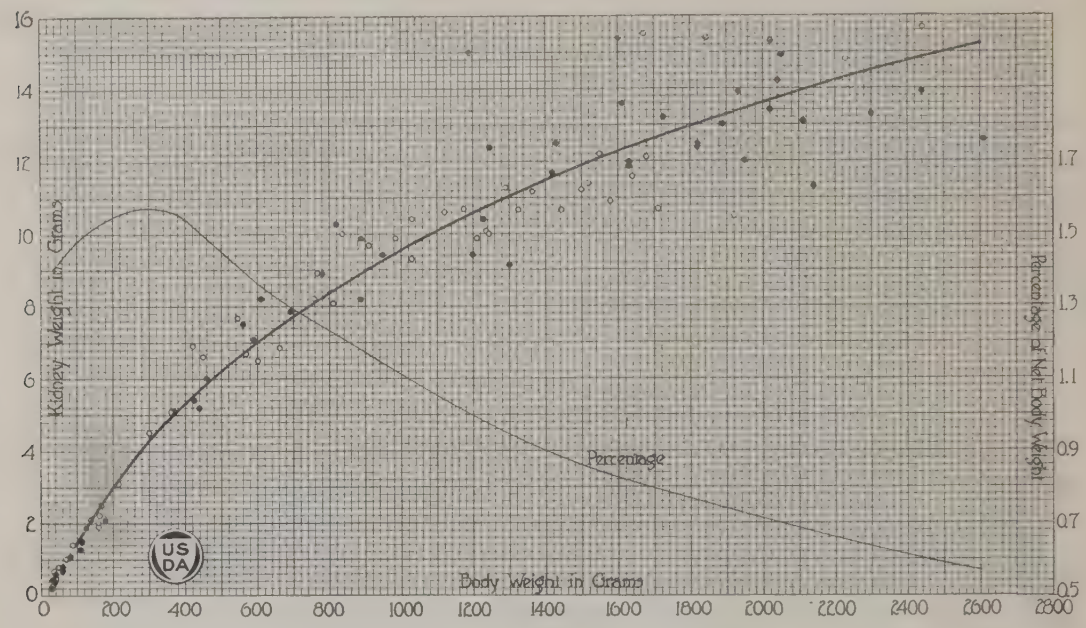

FIG. 26.—Absolute and relative weights of the kidneys, plotted on gross body weight

In the pullet at 230 days there was an interesting example of what appeared to be an ovum which was being resorbed. The yolk mass had apparently been partly resorbed until the membrane was not tense, as it usually is, but flabby. A similar condition was seen in two of the hens.

It is interesting to note that the ovary and oviduct are closely correlated in weight, both increasing or decreasing proportionally.

The greater weight of the ovary in the pullet at 286 days and in one of the hens may be due to the presence of a fully mature ovum, just ready to emerge from the ovary.

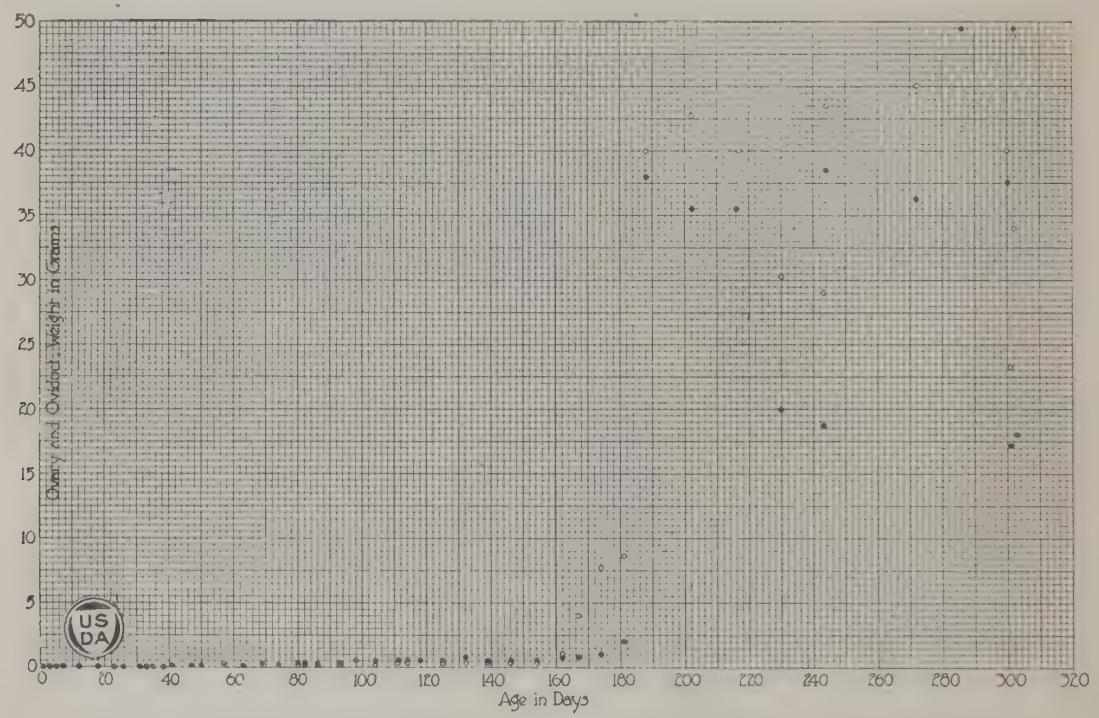

FIG. 27.—Weights of the ovaries and oviducts, plotted on age in days. The solid dots here represent weights of the ovaries. The circles represent oviducts. Both the ovary and the oviduct of one of the hens were too heavy to represent on the chart. The weights were: 68.5 gm. for the ovary and 50.7 gm. for the oviduct. No curves were made, since the cases are too irregularly arranged

The increase in the diameters of the ovarian ova was also recorded, and it apparently is a more gradual change

than is indicated in the change in weight as shown in Figure 27, for the diameter of the largest ovum increases slowly while at the same time there is almost no increase in the weight of the ovary. The first noticeable increase in size of the ova was on the free or ventral surface of the ovary. No measurements were made to verify this observation, for only the largest ovum was measured for each ovary. (See Table II.)

In every female but one (the three-day chick) only the left ovary was found, and no trace of the right ovary was seen. As stated above, the suprarenal on the left side is more and more enveloped by the ovarian ligament as the ovary increases in size, but on the right side the suprarenal is covered by only a thin capsule of connective tissue. In the three-day chick there was a small mass of tissue (not weighed) ventral to the right suprarenal which looked as though it might be a degenerating ovary. Unfortunately, it was not preserved for histological study.

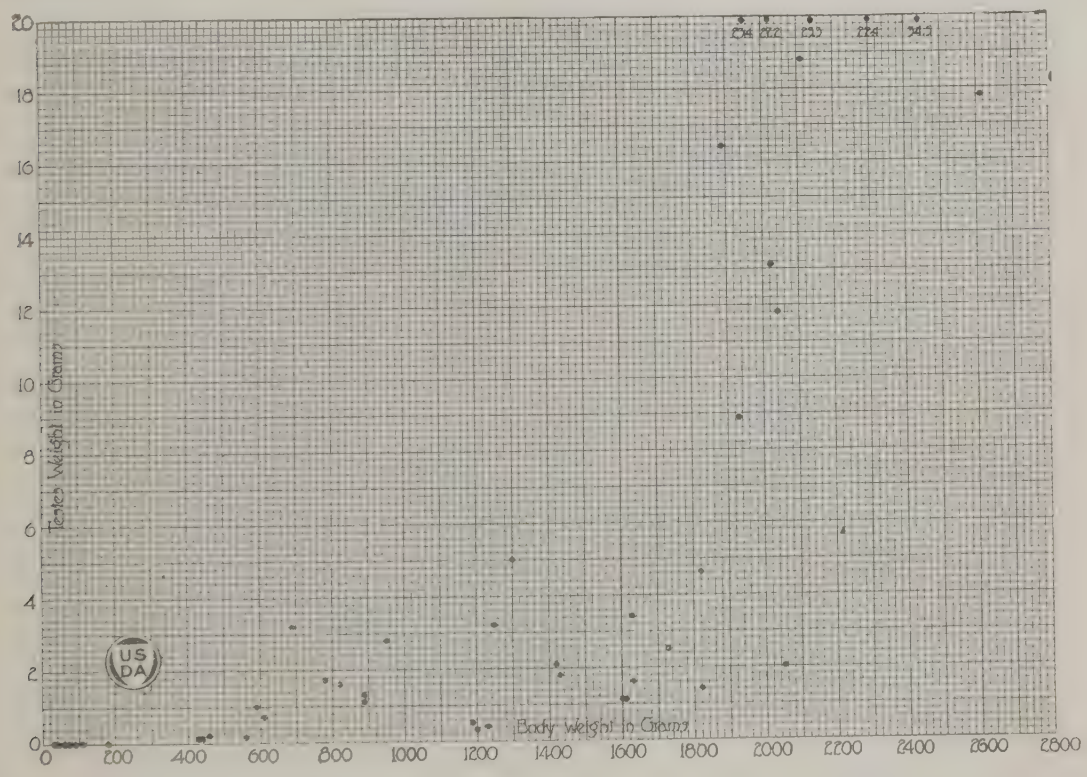

FIG. 28.—Weights of the Testes (in grams), shown as solid dots, plotted on gross body weight. The five cases in which the weight of the Testes exceeds 20 gm. are shown at the upper margin of the chart, and the proper weights indicated. No curves were made, since the cases are too irregularly arranged

TESTES.—The growth in weight of the testes (without epididymis) plotted against gross body weight is shown in Figure 28. Here also no attempt has been made to derive a formula or draw a curve, for the cases are too few in number and too irregularly distributed. There are, however, definite indications of the four-phase curve found in the growth of the mammalian testes. There is (1) a period of slow growth up to 400 gm. in body weight (or about 50 days); then (2) an increase in growth from 400 to 700 or 800 gm. in body weight (50–80 days); and then (3) a period of rapid or puberal growth from 1,800 to 1,950 gm. in body weight (210–260 days); and (4) a terminal plateau or period following sexual maturity. (This last period does not show well in the accompanying chart.)

Autopsy of the males showed an apparent difference in the size of the two testes, consequently the right and left testes of each male bird (with three exceptions) were weighed separately and the sum of the two testes plotted in Figure 28. Up to 179 days the left testis is usually heavier. In 29 specimens under 179 days old the left testis was heavier, and in 4 the right was heavier. Beginning with the one hundred and seventy-ninth day the right testis was heavier in 9 birds and heavier on the left in 4 specimens. Up to 179 days the sum of the weights of the right testes of all specimens was 84.7 per cent of the sum of the weights of the left testes. From the one hundred and seventy-ninth day the sum of the weights of the left testes in all specimens was 91.3 per cent of the sum of the weights of the right testes. The seven males in the in-

anition series (15 culls or malnourished chickens from the same source and autopsied in similar manner) show the same condition. They are all under 179 days of age and the sum of the weights of the right testes is 83.9 per cent of the sum of the weights of the left testes. There is a great deal of variation in the weight of the two testes from the same specimen, for in one male (inanition series) they are identical in weight, while in the 179-day-old male the right testis weighed 1.685 gm. and the left weighed 0.894 gm., or 53 per cent of the weight of the right testis. Further evidence is needed to show whether there is any fundamental relation between the development of the left ovary and this apparently larger left testis up to the time of maturity, and then the more rapid growth of the right testis. Riddle (*21*) finds the right testis larger in both young and adult pigeons of pure species. In hybrids he more frequently finds the left testis larger.

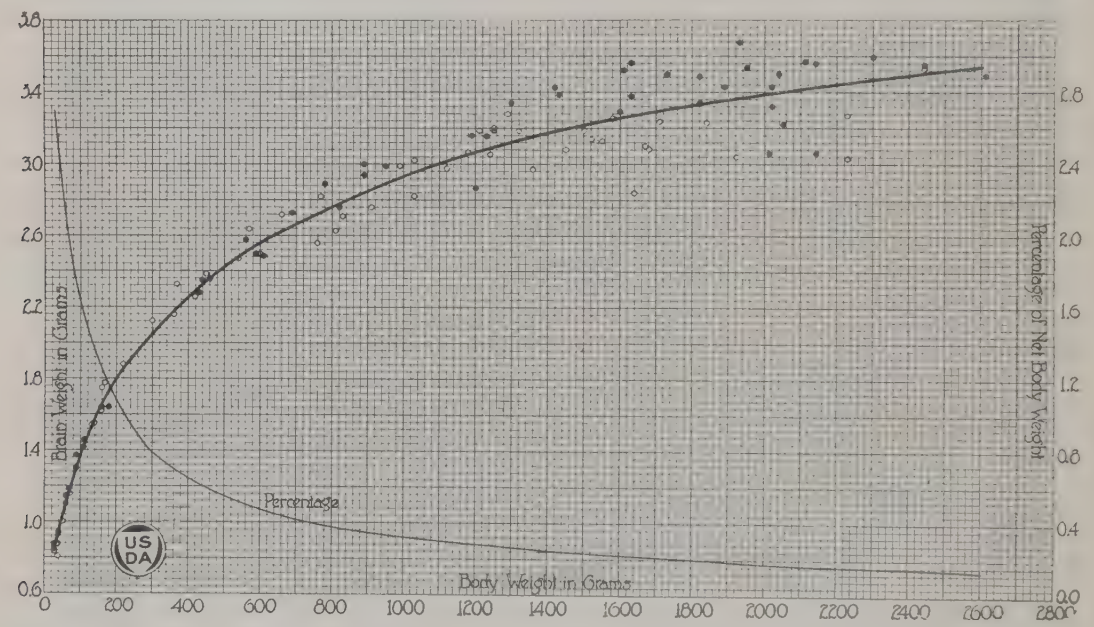

FIG. 29.—The heavy line shows growth of the brain in grams, and the lighter line shows percentage of the net body weight, plotted on gross body weight

NERVOUS SYSTEM AND SENSE ORGANS

BRAIN.—The same precocious growth which characterizes the development of the brain, spinal cord, and eyeballs in mammals is found in the chick. Figure 29 shows the absolute and relative growth of the brain plotted against gross body weight. The formula is:

$$Y = X^{0.22} - 0.00028X - 1.36$$

from 40–2,600 gm. gross body weight.

Y represents the weight in grams of the brain and X the gross body weight in grams.

The rate of growth is very rapid at first and gradually decreases as the body weight increases. There is no significant sex difference, although there is, as in other organs, an apparent difference when the brain weight is plotted against age (due to the sex difference in gross body weight in the later periods).

The percentage weights of the brain show no initial rise but a decrease from about 2.7 per cent at time of hatching. The decrease is rapid at first, or until it reaches about 0.6 per cent; then there is a very slow decrease until it reaches about 0.15 per cent, which continues as the percentage weight for the brain of the adult chicken. Welcker and Brandt (*28*) find that the brain forms 0.24 per cent of the body weight in the two male chickens. This is but slightly heavier than the relative brain weight for the older males of the present series.

Jackson (*8*) states that in the rat the maximum relative weight of the brain is attained at seven days, after which it decreases. The curve of brain weights in the rat, plotted against body weight, as shown by Donaldson (*3*), is very similar to that of the chick as shown in Figure 29.

SPINAL CORD.—Figure 30 shows the absolute and relative growth of the cord, plotted against gross body weight. The formula is:

$$Y = X^{0.14} + 0.00074X - 1.62$$

from 90–2,300 gm. gross body weight.

Y represents the weight in grams of the spinal cord and X is the gross body weight in grams. This curve does no

show the rapid growth during the smaller body weights to so marked a degree as does the curve of brain growth. There is in this case an apparent sex difference (of doubtful significance) at the upper end of the curve.

The relative weights of the spinal cord show no initial increase (as found in the rat). At time of hatching the cord forms 0.5 per cent of the body weight. This decreases rapidly at first, then more slowly until it reaches about 0.12 per cent.

Welcker and Brandt (*28*) give 0.13 per cent for the relative weight of the spinal cord in two adult male chickens. Both the brain and the cord are relatively heavier in the rat than in the chicken.

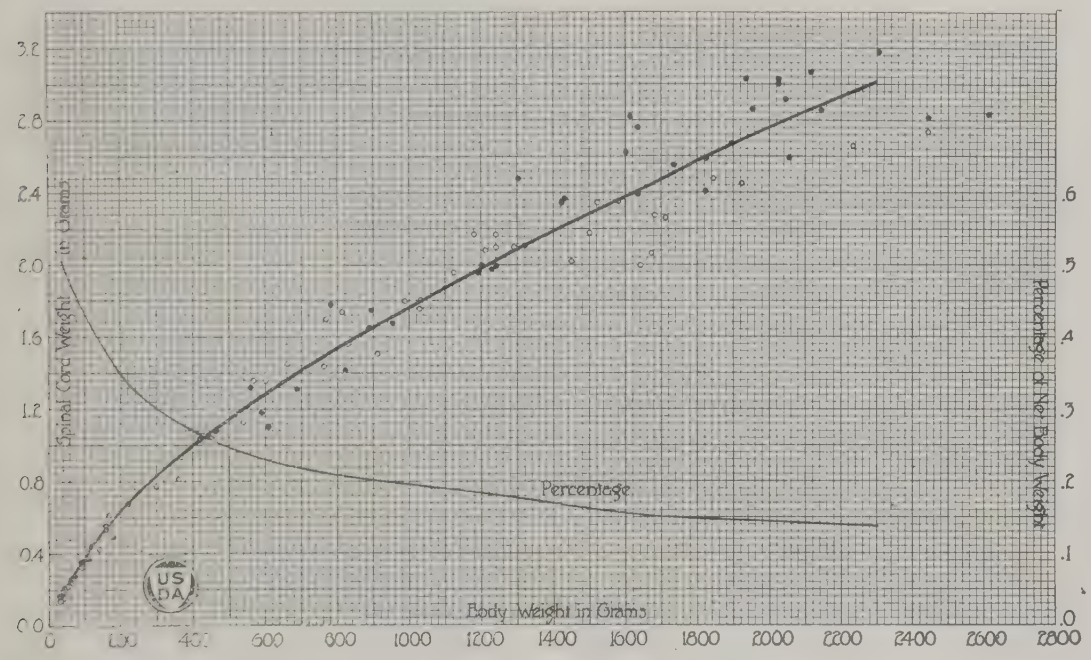

FIG. 30.—Absolute and relative or percentage weights of the spinal cord, plotted on gross body weight

EYEBALLS.—Figure 31 shows that the growth in absolute and in relative weight of the eyeballs is essentially similar to that of the brain and spinal cord. The individual cases, however, indicate a greater variability than is found in weights of brain and spinal

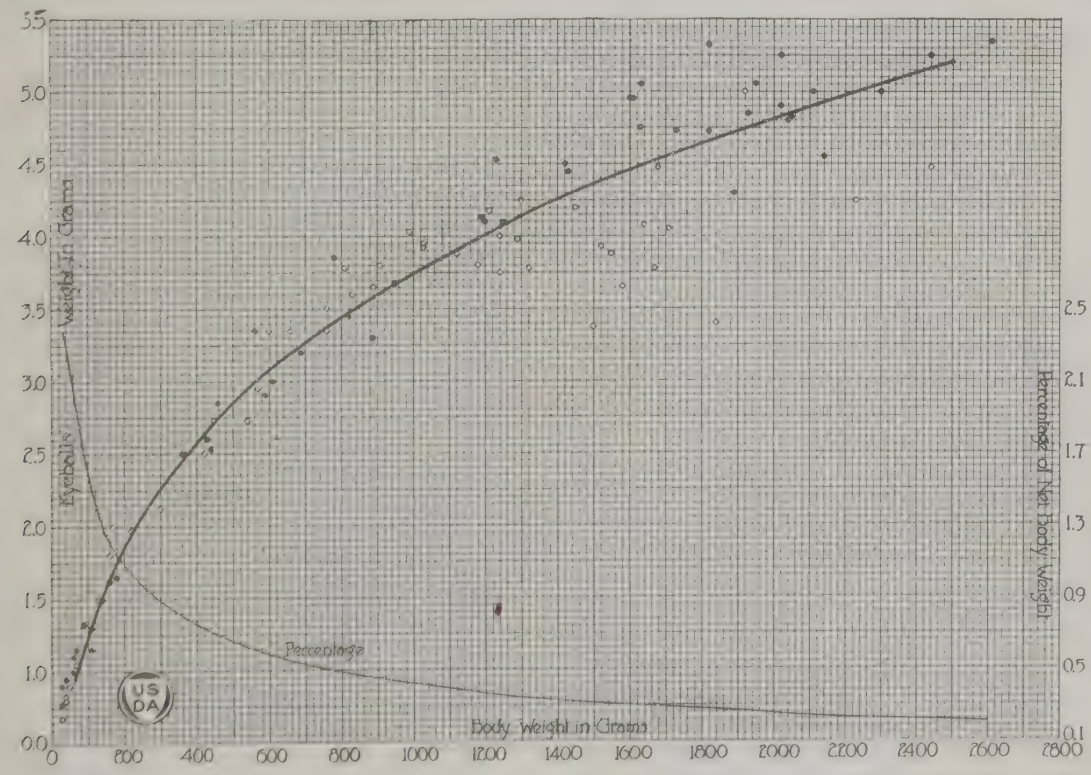

FIG. 31.—Absolute and relative weights of the two eyeballs, plotted on gross body weight.

cord. There is the same indication of a sex difference (of doubtful significance). The formula from which this curve was drawn is:

$$Y = X^{0.251} - 1.91$$

from 100–2,600 gm. gross body weight.

Y represents the weight in grams of the two eyeballs and X represents the gross body weight in grams.

Welcker and Brandt (*28*) found a relative weight of 0.30 per cent for the eyeballs in the chicken, which is a little higher than found in the present series. The three older Minnesota cockerels averaged 0.22 per cent, and the hens 0.21 per cent. The maximum relative weight of the eyeballs in the chick comes at the time of hatching, with a rather strikingly larger percentage weight for the males during the first few days.

DISCUSSION

The growth of the body as a whole in the chicken seems to be somewhat different from that described in man by Scammon (*22*) and Morris.[5] The general character of the curve of growth in the writer's series is similar to that found in earlier investigations on the chicken. This curve shows three phases: (1) a period of slow growth, which includes a brief period of postnatal decrease; then (2) a period of rapid growth, followed by (3) another period of slow growth. This general type of curve holds in the chicken not only for the growth of the entire body but also for the muscles, ligamentous skeleton, digestive tract, lungs, heart, kidneys, suprarenals and integument, when their weights are plotted against age. In man, the growth curve of the body as a whole and of all of the above parts, excepting the suprarenals, is a four-phase curve. The suprarenal in man shows, according to Scammon—

> a great decrease in weight in the neonatal period, an interval from the neonatal period which extends through the greater part of childhood when there is little growth, and a period of rather rapid growth in the prepuberal period and in adolescence.

There is in the chick but slight indication of a slowing of growth, corresponding to the decreased rate of human growth in middle childhood. It is very doubtful whether anything corresponding to the characteristic human prepuberal acceleration of growth occurrs in the chick body as a whole.

When the weights of the organs and systems are plotted against gross body weight instead of age, the first period of slow growth is usually not observed, as shown in the various charts. The exceptions to this are the curve for the thyroid, which is distinctly concave upward throughout its entire length; also those for the muscles and integument, which are nearly straight, and those for heart and lungs.

Another type of curve described by Scammon (*22*) for the growth in the weights of the human brain, spinal cord and eyeballs is similar to the curves for the same organs in the chick when the weights are plotted on either age or gross body weight. All of these curves rise rapidly at first and then more slowly flatten out.

The type for the human thymus with an initial rise up to the age of puberty followed by a decrease in weight is, according to Hammar, characteristic for mammals in general. The present study shows that it holds likewise for the chick. It is also the type of curve found for the growth of the feathers, a fact apparently hitherto overlooked. As above explained, however, the decrease in the weight of the feathers is due to a process quite different from the involution of the thymus.

The growth of the testes of the chicken shows (1) an initial period of but little change; (2) a slightly increased rate of growth followed by (3) a second period of slow growth and of marked irregularity, then a period of very rapid prepuberal growth and finally (4) a period of slow growth. The cases are too few to make a smooth curve but they indicate the above four phases. This is similar to the curve of growth for the human genital organs, excepting ovary and uterus.

The ovary and oviduct of the chicken seem to grow slowly until the very rapid prepuberal growth, after which there is a period of but little growth. The oviduct is lighter than the ovary up to the prepuberal rise. The number of cases is insufficient to warrant final conclusions concerning the growth of the ovary and oviduct, and a comparison of their growth with that in other forms is therefore omitted.

The curves of the growth of the body as a whole show a sex difference apparent very soon after the beginning of the second or rapid puberal growth phase of the curve. This means that at any age after 8 weeks the male chickens average heavier than the females of equal age.

The linear measurements of the body also show a marked slowing of growth in the female earlier than in the male. This means that the female skeleton ceases to increase in length earlier

[5] MORRIS, H. MORRIS'S HUMAN ANATOMY. Ed. 6, Sect. 1. 1921.

than does the male skeleton. A similar difference in extent of growth is true for other organs, but this does not necessarily mean that these organs are proportionally heavier in the male of the same body weight. Plotting these organs against gross body weight usually shows clearly that they are relatively of about the same weight in male and female. As has been mentioned, there still persists a definite sex difference in the weights of the head, skeleton, and feathers and a less marked sex difference in the heart and lungs. The digestive system and all of its parts (except the liver), and the nervous system and eyeballs show a slight sex difference in the upper parts of the growth curves for these organs. By the time this sex difference becomes apparent, the chickens are sexually mature and the females are all fatter than the males. The extra fat thus increases the total body weight perhaps enough to account for the apparent lowering of the weights of the organs in the females, when plotted against body weight. The liver does not follow the same course as the other parts of the digestive tract, for it also apparently becomes loaded with the surplus food material and the fat females therefore also have heavier livers. This is readily seen by reference to Figure 17.

If, however, the surplus fat in the females makes the digestive system and the nervous system appear relatively lighter in the females, it should affect all of the organs similarly. But the suprarenals, thymus, spleen, skin, and kidneys do not show this effect. It is true that most of these curves are more irregular, but at least some indication of the sex difference in weight might be expected, unless some other factor is concerned in this group of organs.

The poultrymen say that chickens two years old should be heavier than at one year of age. Although the writer's cases are very few, it may be interesting to see what changes are apparent in the structure of the older chickens. An actual increase appears in the weights of the kidneys of two of the three hens. The heart shows an increase in absolute and in percentage weight. The thyroid becomes extremely variable, heavier for one and much lighter in two of the females. The amount of fat was not determined accurately, but from inspection it is apparent that a very large part of the change occurring in the second year is due to the increase in the amount of fat. There is an absolute increase in weight of the muscles (possibly due to more fat within them), though they still maintain the same relative (percentage) weight.

SUMMARY

The more important findings may be summarized as follows:

1. The curve of postnatal growth of the entire body of the chicken shows three general phases: A period, first, of slow growth, including a brief postnatal decrease in weight; a period, second, of rapid (pubertal) growth, during which a sex difference in body weight begins; and a period, third, of slow increase in weight.

2. The weight of the head shows a marked sex difference, due apparently to the larger development of the comb and wattles in the male.

3. The growth in weight of the skin (excluding feathers) is directly proportional to that of the entire body, forming about 9 per cent of the net body weight.

The feathers increase in both absolute and relative weights until just before sexual maturity. Then follows a decrease in absolute and relative weights, the growth curve thus somewhat resembling that of the thymus.

4. The skeletal muscles increase from 21 or 22 per cent at hatching to about 50 per cent of the body weight in the adult.

5. The skeleton at first grows a little less rapidly than the entire body. It forms 11 per cent of the body weight in the mature male, and 8 per cent in the female. The weights and linear measurements show that the female skeleton matures earlier than the male.

6. The digestive tube and its regions, stomach (proventriculus), gizzard, and intestines, also the pancreas, all grow at about the same rate, showing a short initial rise in relative weight, followed by a slow decrease up to maturity. The empty tube reaches a maximum of 18.5 per cent of the body on the sixth day, decreasing to about 5 per cent in the adult.

The weight of the "tare" (contents of the digestive tube) is extremely variable.

The yolk sac was found in all chickens autopsied, with one exception, up to and including the thirty-eighth day, and thereafter frequently up to the two hundred and thirty-seventh day. Meckel's diverticulum is constantly present.

7. The liver decreases from an early maximum of 6.2 per cent of the body weight to about 2.5 per cent in the

adult. Its weight increases noticeably in the older chickens, especially in the fat hens.

8. The weight of the trachea and lungs is variable and shows an apparent sex difference in the older chickens, being heavier in the males.

9. The heart shows a marked increase in both absolute and percentage weight during the later part of the growth period, or beginning at about 1,400 grams gross body weight.

10. The curve of absolute weight of the thyroid is concave superiorly throughout. The percentage values are variable with a minimum from 200 to 400 grams gross body weight.

11. The thymus increases in both its percentage and absolute weight up to sexual maturity and then undergoes involution, decreasing in relative and absolute weight. Its changes are more closely related to age than to body weight.

12. The suprarenals and hypophysis are somewhat variable in weight but neither shows a sex difference.

13. The kidneys show a marked initial rise from 0.6 per cent on day of hatching to 2 per cent of the body weight at five days, followed by a slow decrease to about 0.6 per cent in the older and adult chickens.

14. The ovary, oviduct, testes, comb and wattles are extremely variable in weight. They all tend to form a four-phase curve of growth, with a marked acceleration at puberty.

15. The brain, spinal cord, and eyeballs increase rapidly at first, with a slow growth later. The relative (percentage) weights of these organs show no initial rise, but decrease progressively from time of hatching.

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GERANIUM STEMROT CAUSED BY PYTHIUM COMPLECTENS N. SP.

HOST RESISTANCE REACTIONS; SIGNIFICANCE OF PYTHIUM TYPE OF SPORANGIAL GERMINATION [1]

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INTRODUCTION

Geranium cuttings (Pelargonium) are often affected with a blackening and decay of the roots and stems, which may result in complete rotting of the young plant. During the latter part of 1919, *Pythium de baryanum* and three other fungi belonging to the same genus were isolated from blackened geranium stems in the agricultural greenhouses at Washington, D. C., and were found capable of reproducing this condition in healthy cuttings. This paper is an account of one of these isolations, which differed very markedly in morphological and cultural characters from *P. de baryanum*, previously reported by Peters (*14*)[2] and recently by Buddin and Wakefield (*5*) as causing a geranium stemrot. It was further frequently characterized by the stimulation of a definite resistance reaction on the part of the host, evidenced by the formation of a cork cambium within and across the stem at some point in advance of infection, barring further progress of the hyphæ after infection and rotting had already proceeded some distance from the point of inoculation.

SIGNS OF THE DISEASE

The early stage of the disease, as caused by any of the *Pythium* spp. isolated, consists essentially of a progressive blackening and necrosis accompanied by wet rot of noncutinized and nonlignified tissues, usually commencing at the base of the cutting below the ground. Infected plants are not firmly embedded in the soil and offer little resistance to pulling, owing to their inability to form binding secondary roots or destruction of these when already present. General turgidity is not affected at first, so that diseased cuttings may appear normal to the eye until the discoloration has progressed above ground.

After this stage the signs caused by the organism at present under consideration may be distinguished from those caused by the other *Pythium* spp. isolated, by the deeper dead-black discoloration, which progresses much more slowly up the stem and finally stops, usually 20 to 40 mm. above ground. This results in a sharp line of demarcation between the healthy, turgid green tissue and the shriveled black diseased portion below (Pl. 1, A). Under very warm, moist conditions, or when soil nematodes are abundantly present in the rotted tissues, infection may continue until it involves the entire plant, which wilts and rots on the ground. The characteristic limitation of infection is, however, more frequently observed in both naturally infected and artificially inoculated plants, often within six days after inoculation. Cuttings showing this type of infection may remain turgid above the dead portion of the stem so long as four weeks. No further growth takes place, however, and the plants remain dwarfed but turgid until they finally topple over through some external mechanical cause or through the continual weakening of the supporting base. If the stoppage of infection occurs sufficiently near the surface of the ground, secondary roots may be put forth just above the line of demarcation.

A more detailed examination of a diseased cutting in the early stage of infection shows a narrow gray to brown advancing margin, involving all stem tissues. Below this the discoloration is a deeper brown to black, and is accompanied by loss of turgidity in pith and cortex. These tissues are crushed in

[1] Received for publication April 16, 1924—issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 419.

through the release of centrifugal pressure on the epidermal layers, and are rapidly hollowed out by a soft, wet rot. The cutinized epidermis, the underlying cork layers when present, and the lignified elements of the fibrovascular system are not included in this decay but form a hollow double cylinder, the outer (epidermal) firm and continuous, the inner (vascular) shredded and fibrous. Rotting of cortex and pith finally stops at the point of demarcation. Leaf scars included in the diseased area are hollowed out, leaving holes in the epidermal cylinder, through which a black exudate from the decaying tissues may be squeezed out.

Microscopic examination reveals hyaline, nonseptate hyphæ ramifying within and between the cells of the discolored tissues. Oospores with the peculiar antheridia characteristic of this organism are found within the infected cells just before their turgidity is destroyed, and are particularly abundant in the crushed cells of the later stage. Bacteria and nematodes are often present in later stages in the decaying tissue, but not in the turgid advancing brown area in which the coenocytic hyphæ may be observed reaching into the healthy cells beyond.

ISOLATION OF THE CASUAL ORGANISM

The causal organism was first isolated in November, 1919, from two out of six blackened geranium stems collected in the agricultural greenhouses. The remainder yielded *Pythium de baryanum*, which had been isolated earlier in the work and appears to be the pathogen most frequently associated with the stemrot. The fungus under discussion has since been repeatedly obtained and recognized in platings from naturally infected cuttings from Washington and from collections made at Enid, Okla. (1923).

Stems from which isolations were to be made were first scrubbed free of adhering soil, then rinsed in alcohol and immediately flamed. They were then bisected longitudinally downward, beginning at the healthy portion above the advancing margin. A preliminary split was made with a sterile scalpel and the two halves were pulled apart with sterile forceps, thus exposing infected internal tissue untouched by any outer agent. Bits of tissue at the infection margin were quickly excised with a flamed scalpel and planted on corn-meal agar plates. Isolations were also made by searing the surface of the cleaned stem with a hot knife and digging underneath with a flamed scalpel or needle. The first method was usually found more satisfactory, since one could see and select transplant material sufficiently remote from the badly decayed interior to minimize contamination by secondary organisms.

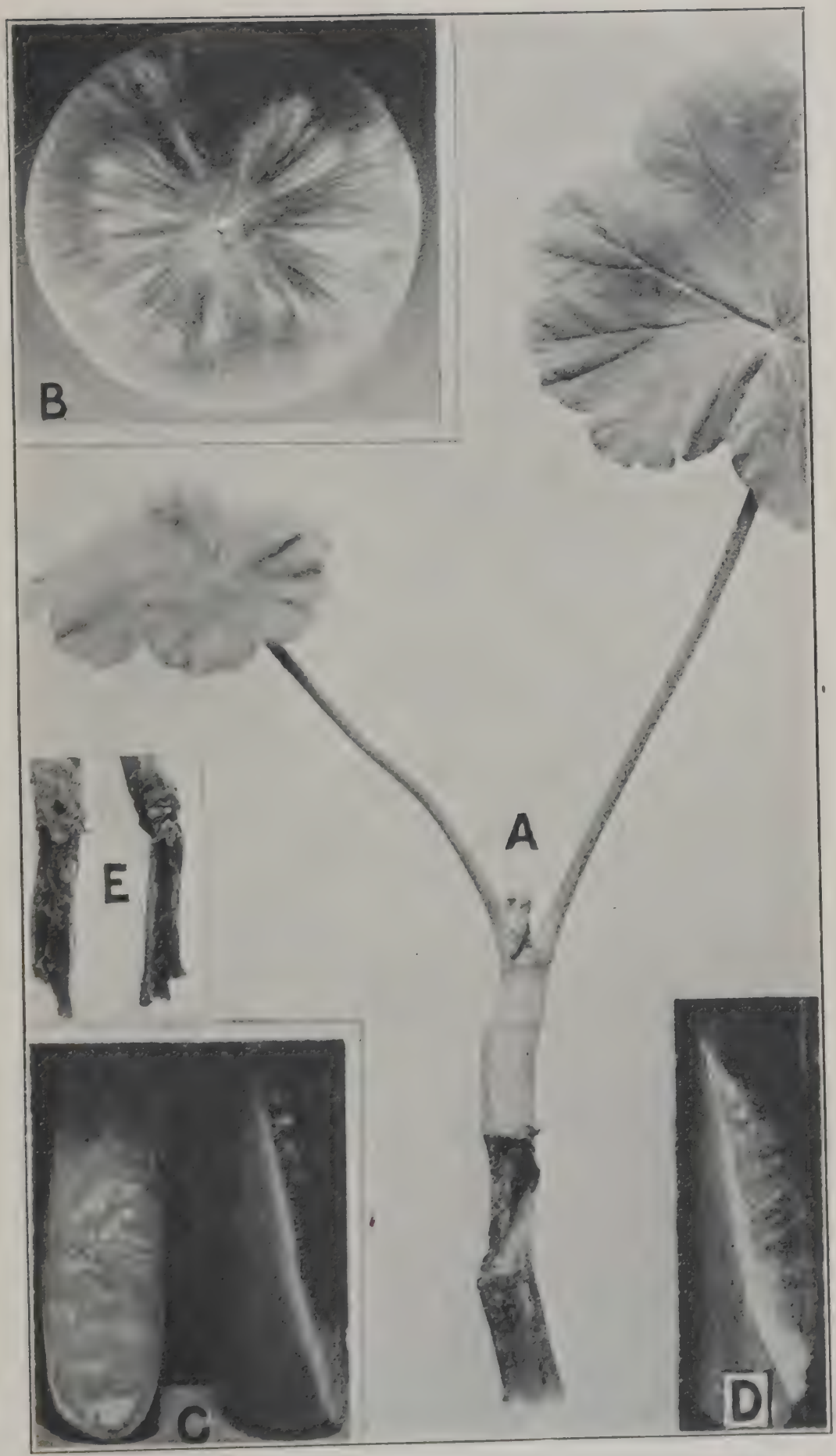
Isolations on corn-meal agar from slightly discolored advancing areas usually yielded colonies which for this organism were characterized by a compact prostrate growth of closely parallel silky hyphæ, later forming an abundance of oospores immediately around the transplant material. The fungus was obtained with difficulty from stems which were hollowed out up to the demarcation; the plates were often overrun with bacteria, nematodes, and soil fungi. In the absence of other fungi, the Pythium could be freed from bacteria by planting part of the growth in the center of another corn-meal agar plate, to which a drop of 50 per cent lactic acid had been added. Transfers were finally made to oatmeal agar tubes for use as stock cultures, after purity had been assured by the absence of bacterial growth in transfers on beef agar plates.

INFECTION EXPERIMENTS

Inoculations have been repeated at various intervals during the past four years, and have shown that the typical blackening and decay can be produced in healthy geranium cuttings when a portion of a pure culture is placed in contact with any wounded part of the stem. Mycelium from oatmeal agar or corn-meal agar cultures was generally used. Inoculations without previous injury were not successful. Since natural infections appeared to progress from the base, it was concluded that the freshly cut base of the stem placed in the soil was the usual means of entry of the organism. Cuttings placed in sterilized soil and inoculated at the base showed within 24 hours a sinking in of the pith and a

EXPLANATORY LEGEND FOR PLATE 1

A.—Geranium cutting eight days after artificial inoculation. Note demarcation at node, shriveled black area below, turgid healthy tissues above
B.—Colony on corn-meal agar plate, natural size, 48 hours at 25° C.
C.—Carrot agar cultures one week old. Front and side views
D.—Potato dextrose agar culture one week old. Side view
E.—Decayed base, advanced state; hollowed out, epidermis and F. V. B. remaining



Geranium Stemrot

Plate 1

(For explanatory legend see p. 400)

brown discoloration and soft rot which spread upward with decreasing rapidity, finally stopping within six to eight days at some point 20 to 40 mm. up the stem. Below this point the decay continued until the epidermal cylinder and the fibrovascular system alone were left. Oospores could be found in infected tissues within four days after inoculation. Reisolation of the organism and successful inoculations with the reisolation completed the pathogenicity cycle. In checks where the severed surface was not inoculated a callus and roots soon formed, binding the plant into the soil, in sharp contrast with the loose condition of inoculated plants.

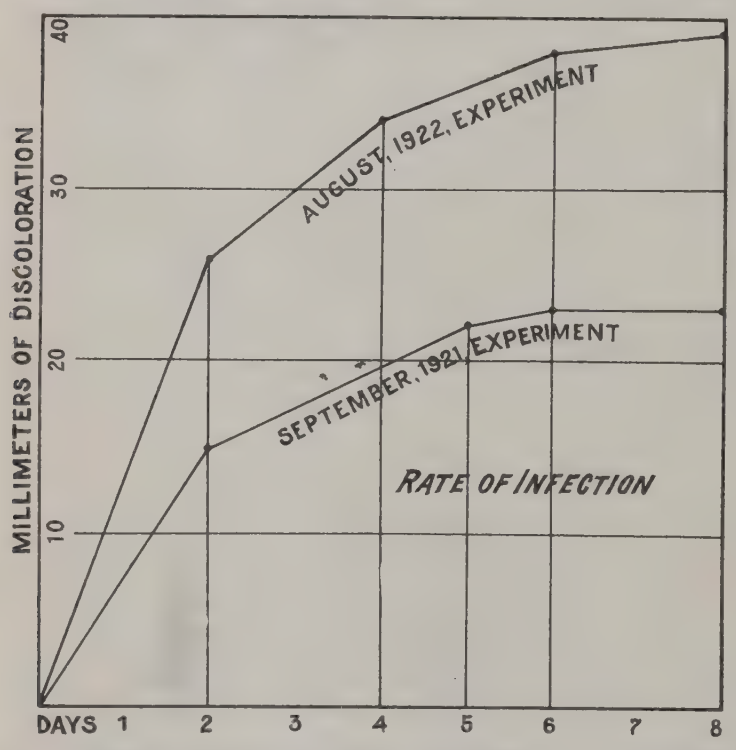


FIG. 1.—Graph showing progress of discoloration of inoculated geranium cuttings, 2 experiments, averages of 9 and 10 plants, respectively

In one experiment in which cuttings were placed in pots which had previously contained artificially infected plants, two out of five developed the stemrot, and the typical oospores were found in the tissues, indicating the rôle of infected soil as the carrier of inoculum. The large number of oospores usually formed and set free by decay of the host cells is doubtless a fruitful source of infection. Since their germination has not been observed, however, even after three months, it seems more likely that the primary source of infection lies in the mycelium and sporangia in decayed tissues, although the latter are not formed so abundantly as the oospores.

Cross-inoculations on cucumber, water cress, and radish seedlings in pots and in sterile tube cultures were unsuccessful, except that superficial black streaks appeared on the radish stems without progressing much farther or causing any wilting. Infection was obtained on Coleus cuttings, which were rapidly discolored and subjected to a shriveling dry rot without any stoppage of infection as in the case of geranium cuttings.

Measurements of the progress of infection in the latter were made in two experiments, including 9 and 10 infected plants, respectively, and are plotted in Figure 1.

SUMMARY OF INOCULATION EXPERIMENTS

DEC. 3, 1919.—Twelve cuttings inoculated at the surface of the ground, six of these without wounding; five checks. All the plants were covered with bell jars. No infection after three weeks in checks and uninjured cuttings, callus and roots forming normally; discoloration and decay followed wound inoculations, rotting entire base but stopping above ground within six to eight days. Reisolations made December 8 yielded 11 similar colonies of slow-growing type with combed-silk effect, like original. Typical oospores appeared on all the colonies.

JAN. 6, 1920.—Six cuttings inoculated at surface of ground, with slight scalpel injury; 10 inoculated at freshly cut base; six checks. Spreading discoloration visible the next day, except in two inoculated at surface, in which the inoculated area dried up and remained uninfected. By the eighth day decay had progressed up the stem from basal infections and had stopped with sharp demarcation. Lateral infections spread in both directions, destroying tissues below. The checks were healthy and formed roots.

FEB. 16, 1920.—Four cuttings inoculated with one of the December 8 reisolations. All blackened on fifth day 8 to 22 mm. up to the stem; no further progress after ninth day; oospores abundant in the tissues. One check. This remained healthy.

MAR. 3, 1920.—Ten cuttings inoculated at base with original isolation. Eight infected; blackening progressed 11 to 20 mm. by fifth day. Further advance very slow, stopping at eighth day. Two inoculated plants and four checks remained healthy.

MAR. 20, 1920.—Five fresh cuttings planted in pots previously containing soil and débris of infected plants of March 3; no direct inoculation made from cultures. Two cuttings planted in fresh pots as checks. Two of the former showed typical infection on sixth day; oospores present. Remainder healthy.

SEPT. 15, 1921.—Ten cuttings inoculated at base. Nine infected. Progress of discoloration shown in Figure 1. The three checks remained healthy.

AUG. 17, 1922.—Ten cuttings, basal inoculation; soil sterilized three times. Sunken base within two days; for discoloration progress see Figure 1. Sharp demarcation eighth day; pith and cortex rotted. Typical colonies obtained in reisolations made August 22. Five checks. These remained healthy.

JULY 26, 1923.—Thirty inoculated. Ten plants (set A) were placed on moist sand for two days before inoculation. The remainder (set B) were inoculated immediately on removal from stock plants. All examined a month later, August 26. Seven of set A healthy. Four of set B completely rotted down; remainder showed sharp demarcation, still alive and turgid, over hollow blackened stem below; epidermal cylinder intact, fibrovascular bundles forming a shredded inner cylinder; one plant had three adventitious roots starting from above the demarcation, which took place just above ground. Diseased areas 20 to 45 mm. long. Abundant typical oospores in tissues. The 10 checks remained sound.

SEPT. 24, 1923.—Three Coleus cuttings inoculated. The inoculated plants rotted completely within five days, without demarcation. The one check remained healthy.

OCT. 18, 1923.—Seedlings of water cress, cucumber, and radish in duplicate 6-inch pots inoculated with and without scalpel injury. No infection in any after two weeks.

FEB. 2, 1924.—Seedlings of cucumber and radish in glass moist chamber were inoculated, without injury. There was no infection on cucumbers. Black streaks appeared at points of inoculation on radish stems without progressing much or affecting turgidity.

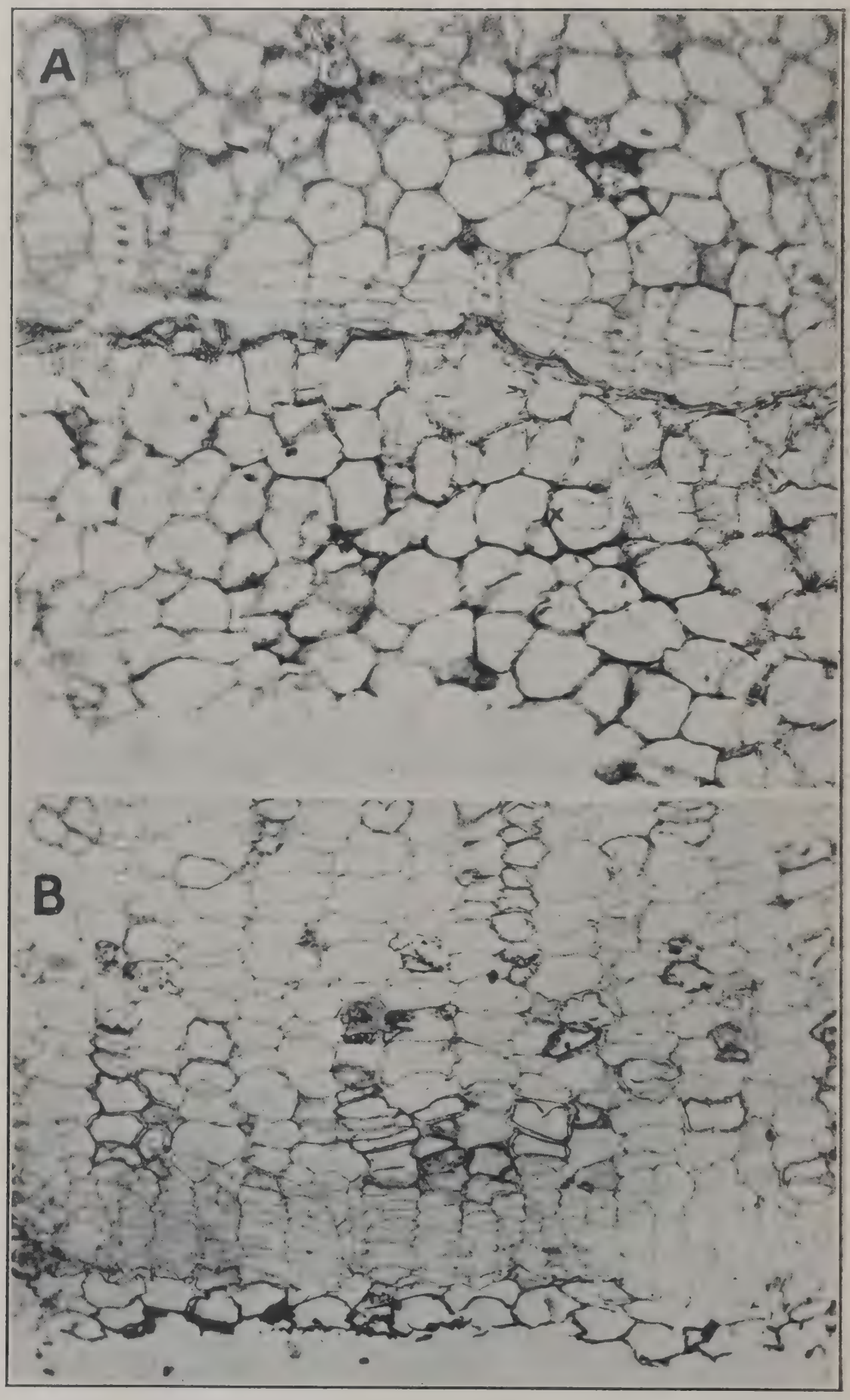
FEB. 16, 1924.—Seedlings of cucumber and radish (six each) grown under sterile conditions on Knopf's agar in test tubes, inoculated with and without wounding. The mycelium made some growth on the agar, but the cucumbers remained turgid and were not discolored. Black streaks not present in the checks appeared on radish stems but without affecting turgidity.

PATHOLOGICAL HISTOLOGY

The hyphæ are largely intracellular and show a constriction when passing through cell walls. They are found one or two cell layers ahead of cells with discolored walls, indicating that there is no lethal action in advance or that penetration is more rapid than visible degeneration and discoloration. Branched coils and nests of hyphæ may be observed within the older infection area. Long strands run within and between sieve tubes and companion cells, with branches reaching out into cortex and pith. Oospores are present in great abundance within cells some distance back of the advancing margin and may be observed in progressive stages of maturation in a single section. Xylem and the cuticularized epidermis are not invaded. Penetration of the subepidermal cork layers from within rarely proceeds farther than the innermost, youngest, and least suberized layer.

Infected cells are killed immediately or very shortly after penetration, the walls soon taking up the brown discoloration. The thin-walled pith cells are the first to lose turgidity, resulting in the sunken appearance of the pith at the base of infected cuttings. Later the cortex also breaks down. The region of collapsed cells is often sharply delimited from adjoining infected but still turgid tissues (Pl. 2). Both pith and cortex are soon hollowed out by gelatinization and solution of their walls. The host nucleus is discolored but not corroded, and may be recognized for a long time after the cell has collapsed. Starch appears to be as abundant in diseased as in healthy tissues, except as noted below; mature oospores in collapsed cells may often be found among masses of uncorroded starch grains still embedded in their plastids.

Stained sections through the stem at the line of demarcation reveal an interesting condition. A cork cambium 4 to 12 cells thick extends irregularly but completely across, marking off healthy from diseased tissues (Pl. 3). It is usually indented upwards when crossing the fibrovascular bundles, along each side of which it may extend for some distance. The walls of the lowermost cambium layers (nearest the diseased tissues) are collapsed and take the gentian violet in the Flemming triple stain, indicating suberization. This conclusion has been confirmed by microchemical tests with Sudan III, Scarlet Red, alcoholic solution of chlorophyll, iodine and sulphuric acid, alkannin, and iodine. The walls of



Geranium Stemrot

Plate 2

(For explanatory legend see p. 405)

the more turgid upper phellogen cells, as well as the healthy parenchyma above, are deeply stained with the Orange G, indicating their cellulose nature. Starch has completely disappeared for a considerable distance above and below the cork layer. Immediately underneath the collapsed stratum of suberized walls are one to four layers of infected cells, whose walls are still rigid, slightly swollen, often split and pulled apart, and characterized by failure to take up completely any of the stains in the Flemming triple or the iodine green-acid fuchsin combination after fixation with Merkel's or Flemming's Strong. They remain a clear amber color, as in sections of fresh material. Negative reactions were obtained for lignin, suberin, pectin, and cellulose, except that solution took place in concentrated sulphuric acid. The contents are disorganized, and hyphæ (often oospores) are present, reaching up to the suberized layer but not beyond it.

Below this "nonstaining" layer is the remainder of the infected tissue, bordering on the hollowed part of the stem. The walls are swollen and stain deeply with the safranin of the Flemming triple, indicating pectinization. This was confirmed by positive reactions with Bismark Brown, Methylene Blue, and Ruthenium Red. Examination of walls in the "nonstaining" region bordering on the pectinized cells showed in many cases an extension of the safranin along the outer lamina. Thus, a single cell often showed complete reddening of the basal wall immediately adjacent to the pectinized tissues, the remaining walls being amber colored except for a short continuation of safranin on the outer parts of the two walls neighboring on the wholly stained wall. The fact that safranin is taken up only on the surface of the "nonstaining" wall implies action of pectinase from without, and precludes the possibility that it is due to incomplete washing out during the staining process, which would have left the safranin in the innermost lamella. That complete pectinization and solution up to the suberized layer ultimately takes place was shown by examination of cuttings which had been infected for several weeks and had formed the usual demarcation line. Here complete hollowing out had taken place and the suberized layers, now several cells thick, bordered directly on the cavity (Pl. 3).

The formation of a cork layer across the stem acts as an effective barrier to further progress of the hyphæ, which have not been observed above it except where fissures have occurred through the action of nematodes or mechanical injury. It is clearly a specific reaction on the part of this host to this organism, since it was not present in the case of the other three Pythium species studied nor in the case of Coleus cuttings infected with this fungus.

MORPHOLOGY AND DEVELOPMENT OF THE FUNGUS

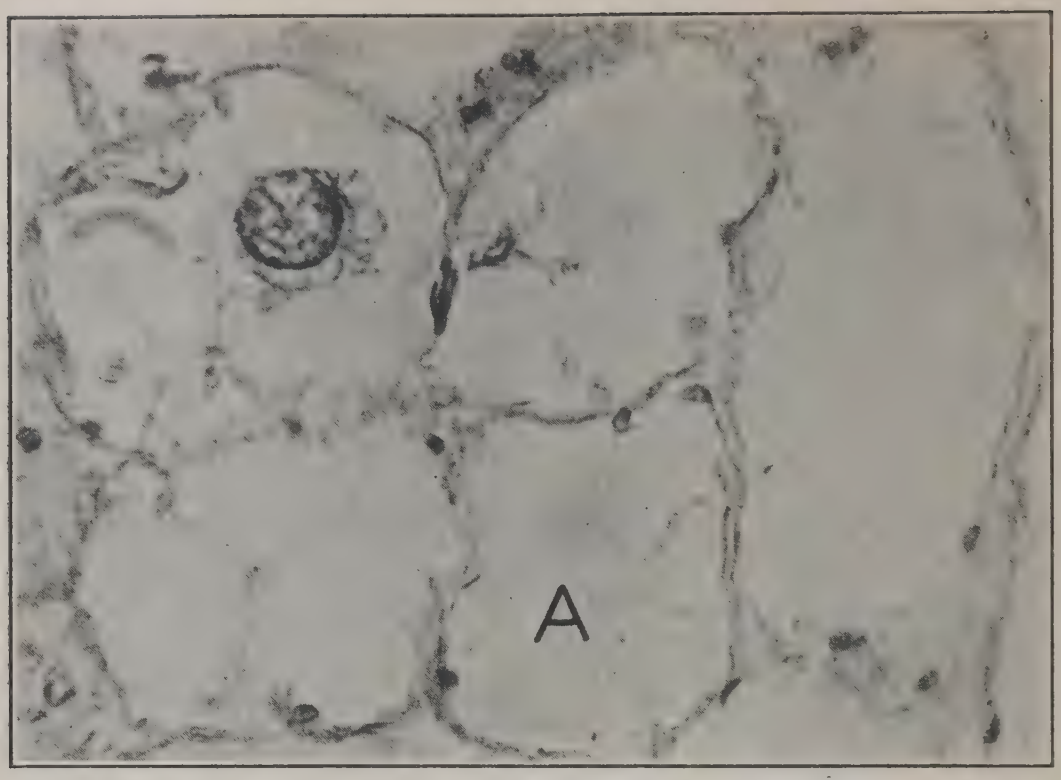
THE HYPHÆ.—Hyaline, coenocytic when young, cylindrical, with no tapering in main or lateral branches (Pl. 4, E). They are very slender, measuring 1.70 μ to 4.85 μ (75 measurements). Branching is abundant and irregular; lateral hyphæ often extend beyond the parent hypha and become main branches in turn. The angle of branching varies from 45° to 90°, followed by a sharp swing forward, which results in a characteristic closely parallel growth on solid media, like combed silk. Smaller subsidiary branches are given off profusely and curve irregularly. A transparent Liesegang-ring effect in the otherwise dense growth has been found to be caused by a comparative scarcity of these smaller branches in these rings, thus affecting the otherwise uniform density of the growth. When growing plate cultures are inverted under the microscope, the hyphæ at the edge of the colony sometimes cease elongation at their original diameter, and put forth a slender prolongation from the tip, which broadens out farther on into the normal diameter and resumes the usual course of growth.

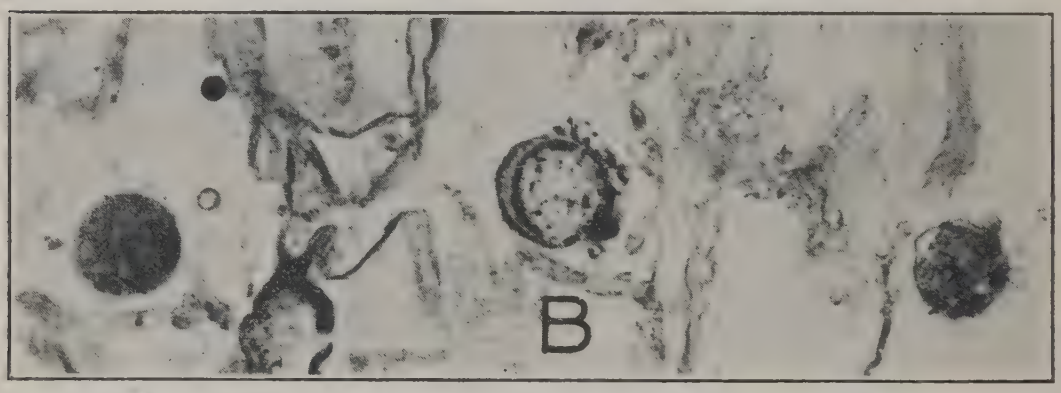
SPORANGIA.—The asexual fruiting bodies are always borne singly. They are usually terminal, less frequently intercalary (pl. 4, F), and sometimes sessile, owing to continued lateral growth of the hyphal tip from the base of the sporangium. Terminal and sessile sporangia are uniformly oval to spherical. Intercalary sporangia are irregularly oval to subspherical, often asymetrical, with flattenings at the places of attachment. Measurements

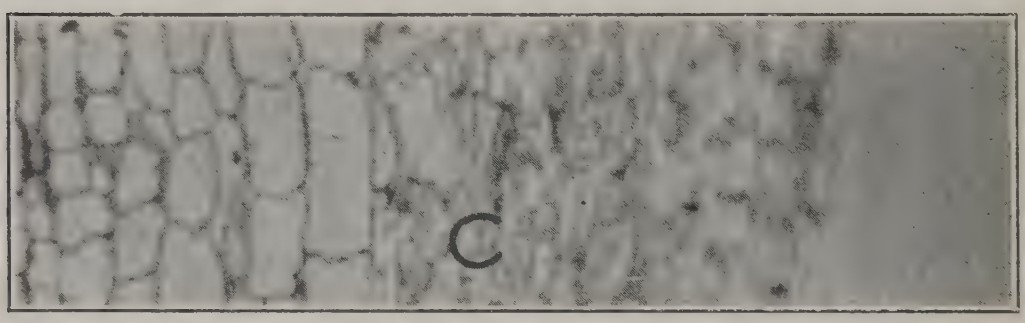
EXPLANATORY LEGEND FOR PLATE 2

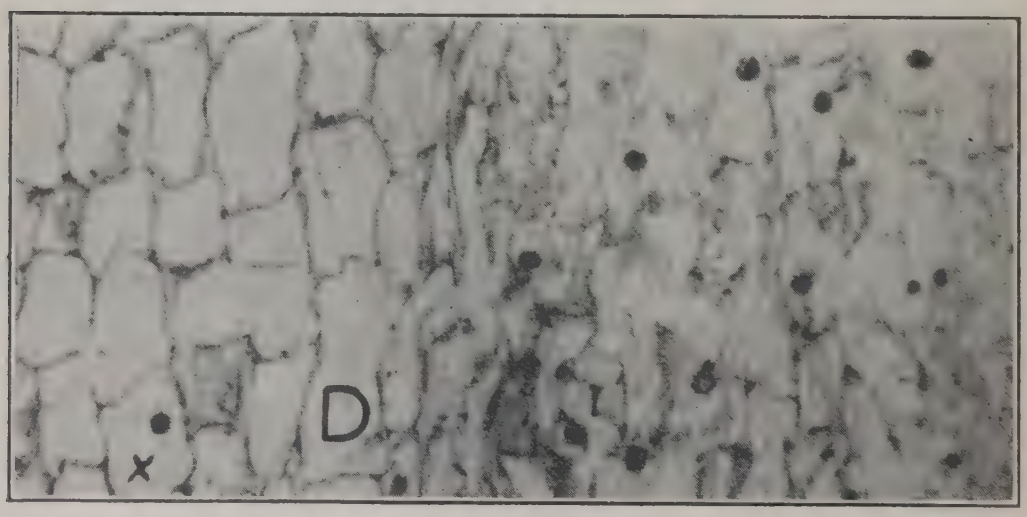
A.—Section of stem at demarcation. Healthy cells at top; cork cambium center; below, single layer of crushed cells, suberized; split and swollen walls of infected cells below, deeply stained; hyphæ (at *x*); pectinization of lower cell walls, causing cavity

B.—Later stage, cambium layers increased; decay of diseased tissues has progressed up to the protective cork cambium









Geranium Stemrot

Plate 3

(For explanatory legend see p. 407)

of 100 sporangia from corn-meal agar plates average 21.85 μ, with ranges from 16.4 μ to 27.26 μ. Carrot agar and corn-meal agar are most favorable for their formation. They appear within four days after inoculation, on the upper surface of the agar, rarely within the agar or at the interface of agar and glass.

The walls are smooth, thin, and hyaline. No papilla has been observed. The contents are hyaline and finely, uniformly granular, with a varying number of small, round, darker spots which are evidently nuclei, judging from comparison with stained sections. Newly formed sporangia are nonvacuolate; older sporangia contain an irregular central vacuole, which increases with age, and occupies one-fifth to one-half the diameter of the sporangium.

The formation of the sporangia is readily observed in inverted blocks of corn-meal agar or in drops of oatmeal decoction, and presents the usual features found in Pythium—the swelling up of a hyphal tip (or segment when intercalary) into a globular body, which is then cut off by a septum, with occasional continued growth of the hypha laterally in the case of sessile sporangia. They are not readily detached, and often germinate *in situ*.

For about 10 days after their formation the asexual fruiting bodies germinate by the extrusion of the undifferentiated contents through a short straight tube into an evanescent, delicately walled vesicle, where the zoospores are differentiated and whence they escape into the surrounding water. As older sporangia are placed in conditions favoring germination, the percentage germinating in this manner decreases, and a greater number protrude a tube, which continues to grow, branches, and forms a mycelium. Both of these methods of germination have been watched repeatedly and are described below in detail (Pls. 4 and 5).

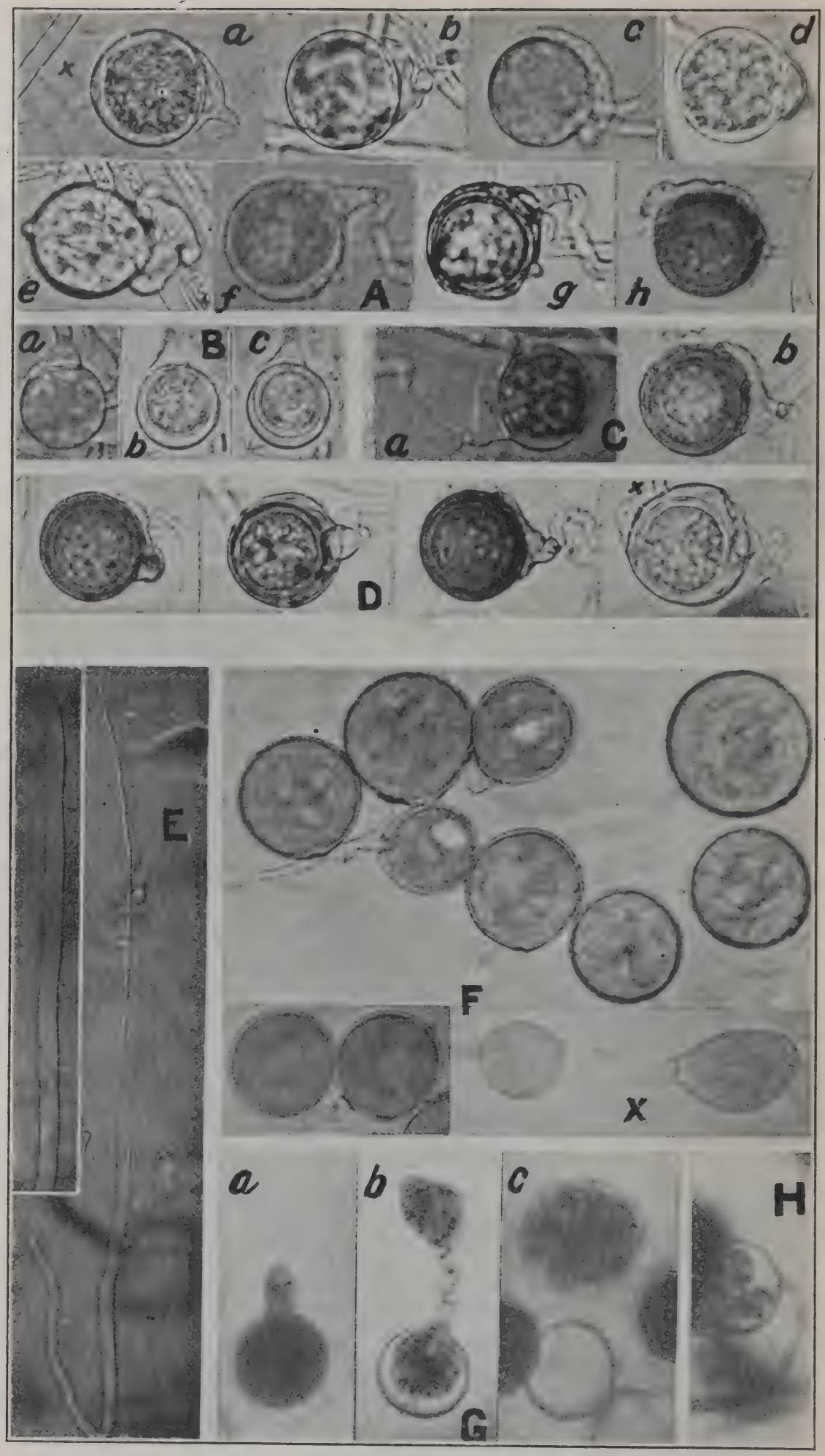
A surface scraping near the point of inoculation of a 6-day-old corn-meal agar plate culture usually yields an abundance of sporangia, which may be placed for observation in a hanging drop of distilled water, previously aerated by a vigorous shaking of the bottle. Within 15 minutes a broad, thin-walled tube is seen protruding from most of the sporangia in the drop. The tube may issue from any part of the surface. Its contents are at first clear and highly refractive; later on granules from the sporangium gradually wander in. During the next 10 to 15 minutes the tube continues a slow growth, which ceases when a length of a third to half the diameter of the sporangium has been reached. In the meantime the sporangial contents have been in slow motion, the vacuole and small dark spots changing their position gradually and irregularly. The tip of the tube suddenly blows out into an expanding bubblelike vesicle, whose wall is barely distinguishable from the surrounding medium; the sporangial contents ooze into it as it expands. The protoplasm within the sporangium breaks away from the wall opposite the tube, leaving lengthening fine strands attached to it. The vacuole, when present, does not appear to enlarge but is included in the general mass squeezing through the tube, and may break if too large.

After the greater part of the contents has entered the bladder, the passage of the remainder gives the impression of a pulling through by the force of surface tension tending to round up the entire protoplasmic mass into the vesicle. Infrequently, part of the contents breaks off and remains within the sporangial wall. Here it differentiates into a very few zoospores which fail to escape (Pl. 4, H) and finally degenerate.

The sporangial wall collapses slightly in places and becomes irregularly angular. The contents are now clear except for a few irregular strands which connected the wall and the viscous departing protoplasm. The latter does not completely fill the vesicle and is at first irregular in outline. It gradually assumes a smooth rounded form through the continued action of surface tension, and presents the same general appearance as when within the sporangium, except that considerably more space is occupied owing to imbibition of water in the absence of rigid confining walls. After a short period of quiescence, clear furrows and wedges appear on the periphery, from which vague lines of demarcation grow in toward the center of the mass. A rocking movement begins and increases in vigor as the lines become clearer and delimit definite re-

EXPLANATORY LEGEND FOR PLATE 3

A.—Oospore and mycelium, in turgid cells. Stained. Oospore contracted, antheridium lobed
B.—Oospores in crushed cells. Stained. Antheridium appressed; oosphere immediately subjacent
C.—Section of pith. Note turgid infected cells, demarcation, crushed cells, and cavity
D.—Oospores in crushed and turgid (at *x*) pith cells



Geranium Stemrot

Plate 4

(For explanatory legend see p. 409)

gions of protoplasm. Cilia may be seen lashing about at the margin, which is now well indented. The general mass increases in volume with the appearance of single vacuoles within the zoospores, which are now well marked off and begin to separate at their tips. They move slightly apart and over each other for a short period, then break apart singly or in entangled pairs and swim away through the disrupted membrane. In one case the first few zoospores to escape left through the same part of the vesicle; but as a rule there is no localized point of exit, the vesicle wall apparently splitting at several places through the impacts of the moving zoospores. In some cases the entire mass of entangled zoospores may break out of the membrane and move off some distance before separation takes place

No trace of the vesicle can be seen after the escape of the zoospores. The open tube, which has acquired enough wall material to insure rigidity, remains on the old sporangial wall. It is now rarely more than one-third the diameter of the spore, and the additional length observed in the early stage of germination evidently had gone to make up the vesicle.

The process of zoospores formation, from the protrusion of the tube to the escape of the zoospores, takes about 15 minutes. It is more or less synchronous in most of the sporangia in a drop, which may be seen swarming with zoospores half an hour after sowing.

Zoospores.—From 10 to 26 are formed from each sporangium. They are broadly lenticular, 5.9 μ to 8.5 μ wide by 10.6 μ to 11.5 μ long, contain a single small round vacuole surrounded by finely granular protoplasm, and bear two cilia at the hilum (Pl. 5, A). After swimming around for half an hour to an hour, they come to rest, and the ciliary motion gradually ceases as the zoospore rounds up. A very slender germ tube is put forth, which broadens back to the base as it grows in length and branches out into a mycelium.

Asexual fruiting bodies from older cultures show an increased tendency to direct germination, particularly in the case of those with large vacuoles. The tubes do not differ at first from those extruded in the indirect process, except that more than one may be formed from a single spore. The tips continue to grow, branch, and form a mycelium.

Oospores.—These are formed in great abundance within the cells of diseased tissues, on oatmeal agar, and geranium agar. They are globose, smooth walled, lie free within the oogonial wall, and average 16.18 μ in diameter (300 measurements), ranging from 11.32 μ to 20.85 μ. The contents are hyaline, with fine granules and oil drops surrounding a large rounded excentric vacuole half the diameter of the oospore. The walls are hyaline when young, later becoming yellow to brown. The formation of discolored oxidation products on substrata is evidently a factor in wall coloration, since oospores from oatmeal and corn-meal agar, which are not discolored, remain light yellow, whereas oospores from host tissue and geranium agar, both of which are browned, are dark walled.

The oogonial wall is smooth or slightly collapsed, and often bears traces of the oogonial stalk.

The most characteristic feature of the mature sexual fruiting body is the antheridium. It is one-celled, persistent, and varies in shape from a trumpet form flaring out at the region of attachment, to a broad irregularly lobed mass clasping or wrapped around a large part of the oogonium and fused with it. The existence of a lateral pressure is indicated by the collapse of that part of the empty oogonial wall immediately underneath. The cylindrical or clavate type so characteristic of most *Pythium* spp. has not been observed in host tissue.

EXPLANATORY LEGEND FOR PLATE 4

A.—Oospore formation, early stages (*a* to *h*). Note clasping antheridium; stalked oogonium (at *ax*)
B.—Three stages in maturation of same oospore (*a* to *c*)
C.—Oospores in contracted oosphere stage (*a*) and exospore stage (*b*)
D.—Mature oospores
E.—Hyphæ, showing cylindrical nature and rounded tips
F.—Sporangia-terminal, and intercalary (at *x*)
G.—Sporangial germination: (*a*), Tube protruded; (*b*), contents partly in vesicle; (*c*), contents extruded, undifferentiated
H.—Three zoospores entrapped in sporangial wall, after most of the undifferentiated contents had emerged

Observations on development and fertilization have been made repeatedly on living material growing in hanging drops of oatmeal decoction, in which the young fruiting bodies appear within two days (Pls. 4 and 5). The antheridium is in contact with the oogonium at a very early stage, before either has been cut off by a septum. At this time it is larger than the oogonium and partly wrapped around it, and presents the characteristic flaring shape. It arises from a neighboring hypha, from an adjacent branch of the same hypha, or immediately beneath the oogonium. The latter is formed by the swelling of a hyphal tip, finally outgrows the antheridium, and both are cut off by septa (Pl. 5, B). Mature oogonia average 18.56 μ in diameter (300 measurements) and range from 13.2 μ to 23.31 μ.

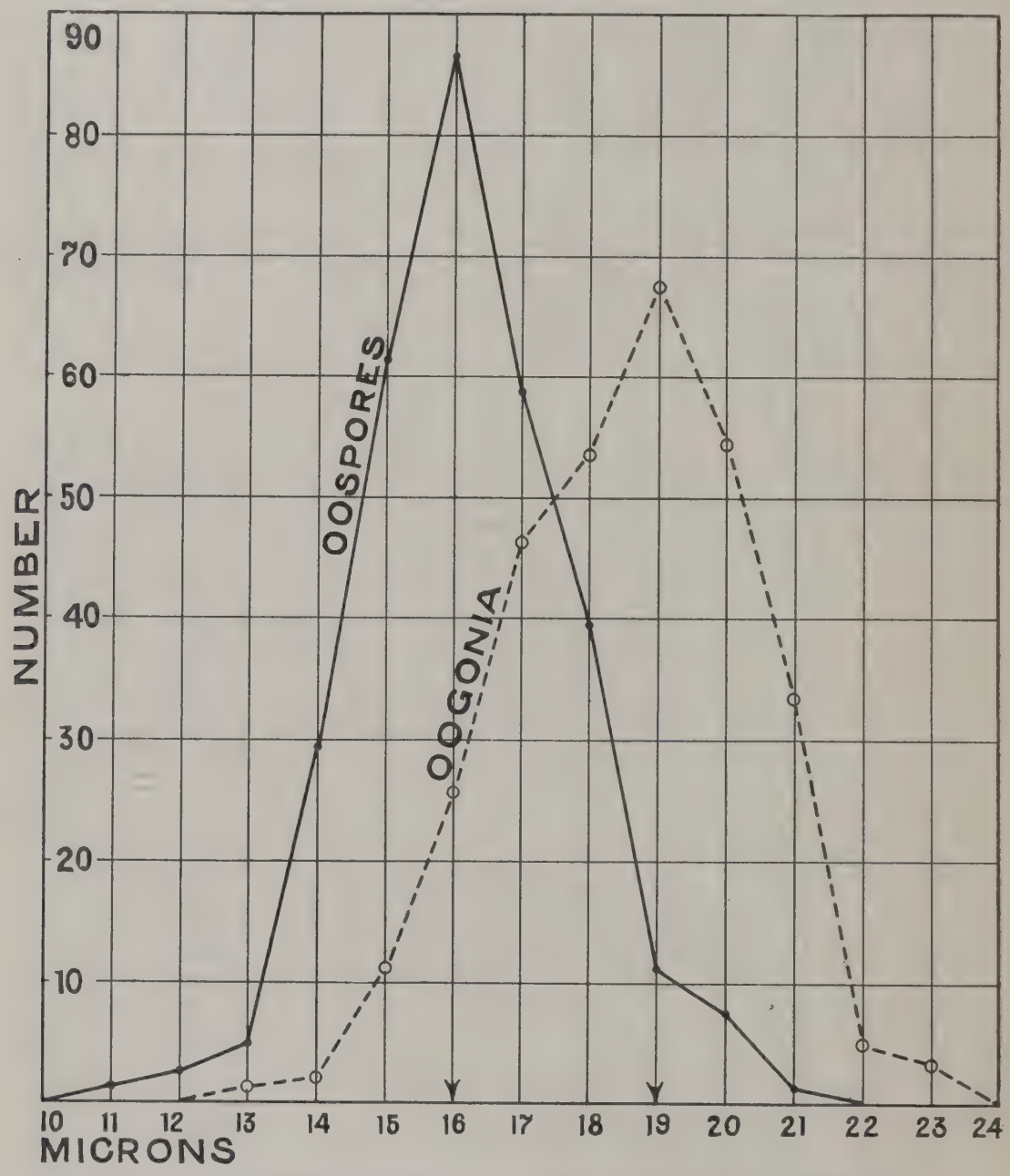
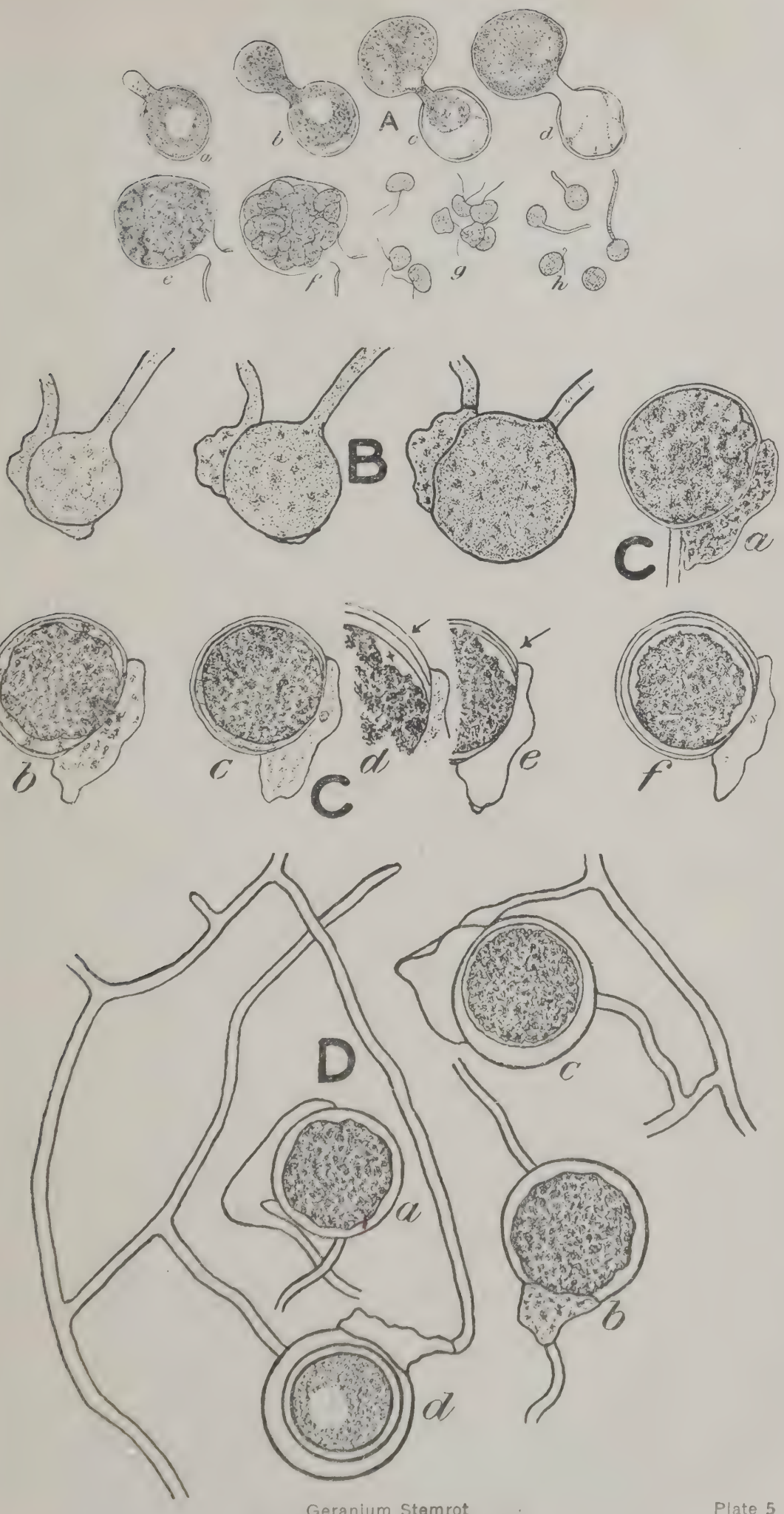


FIG. 2.—Frequency curves of oospore and oogonium measurements, 300 of each

EXPLANATORY LEGEND FOR PLATE 5

A.—(*a* to *h*), stages in formation and germination of zoospores
B.—Three stages in early growth of sexual bodies
C.—Fertilization: (*a*), Beginning of oosphere contraction; (*b*), passage of antheridial contents into subjacent oosphere through hole in fused walls; (*c*), oosphere rounding up; (*d*, *e*, *f*) exospore wall formation by clear band extending around oosphere periphery. The clear band in (*e*) and (*f*) is drawn at twice actual width, to render it visible at scale of reproduction
D.—Oospores, showing attachment on slender stalk and relation to antheridium



Geranium Stemrot

Plate 5

(For explanatory legend see p. 410)

99185—25†——4

The protoplasm in both antheridium and oogonium is nonvacuolate and at first finely granular. The oogonial contents gradually become denser and lumpy and undergo a slow irregular motion, accompanied by the appearance and disappearance of darker particles similar in shape and size to the nuclei observed in stained sections. The antheridial contents have also become coarser but remain much more hyaline. Considerable pressure is exerted by the closely appressed antheridium, evidenced by the depression of the oogonial wall and the consequent irregularity of the otherwise spherical oogonium.

Contraction of the oosphere is initiated by the appearance of irregular clear wedges at the periphery, first visible near the region of attachment to the antheridium and progressing around the periphery until the oosphere acquires a lumpy, irregularly oval shape, separated from the wall by a perfectly clear space except for occasional thin protoplasmic strands. The antheridial contents have also undergone a slow, irregular streaming and appear most hyaline at the base near the septum.

When the oosphere has fully contracted, it is invariably found in close contact with some part of the wall fused with the antheridium. A fertilization tube has not been observed, although dozens of fruiting bodies have been watched at this stage; nor have stained sections so far indicated more than a fusion of antheridium, oogonium, and a flow of antheridial content into the subjacent oosphere, through a break in the region of fusion. In living material a slow emptying of the antheridium may be seen at this stage. In the few cases where fertilization took place in the optical plane a cylindrical mass of protoplasm could be seen slowly progressing from the antheridium into the oosphere through a clear space in the fused wall (Pl. 5, C, *b*). An interface between the two masses of protoplasm was clearly visible, but was wavy and irregular, not smooth, like the fertilization tube of *P. de baryanum*, which was also kept under observation. The antheridium is left empty except for a few strands and a large oily globule at the mouth.

After fertilization the irregularly contracted oosphere quickly rounds up into a smooth ball, slightly increased in volume. Oospore wall formation is initiated by the appearance of a short very narrow clear strip (Pl. 5, C, *d* at *x*) at some point tangentially within the periphery of the oosphere; this extends slowly around the circumference until the contents are inclosed by a narrow clear band, whose outer edge is perfectly smooth and circular, the inner edge being uneven and warty. (Pl. 5, C, *d*, *e*, *f*.) A contraction begins, similar to that of the original oosphere, except that a perfectly homogeneous refractive material is left in the wake of contraction. This proceeds until the full thickness of the wall is laid down, whereupon the irregular periphery of the contents smooths out into a circle. In the meantime the contents have become less lumpy and more finely granular; two dark round bodies may be seen in the center, probably the fusion nuclei; a vacuole appears and enlarges, and minute oily globules appear in the surrounding protoplasm. The antheridium shrinks and remains attached to the oogonial wall as the oospore attains maturity. Oospore germination has not been observed.

CULTURAL STUDIES

Growth of this fungus was studied on 16 media in four culture series, each of which included triplicate inoculations on all media. Cultures were kept in the dark at room temperature (18° to 25° C.) for three months. In the main the terminology suggested by Harsch and Long (*11*) has been followed. Comparisons over a four-year period with the other three *Pythium* spp. have shown a constancy of characters of definite diagnostic value, which has emphasized to the writer the necessity and the feasibility of a standardized procedure and terminology for the study of cultural characters of fungi, such as is available to the bacteriologist. Growth on these media is described below:

Corn-meal agar.—Upper half of slant covered with downy white aerial growth, becoming loose and cobwebby toward base of tube, flattening down after two weeks to form a hyaline, smooth, sodden mat; closely parallel hyphæ at edge of colony, resulting in a striking combed-silk effect when viewed by transmitted light; irregular radiating patches of varying density in older part of colony. Growth on corn-meal agar plates prostrate and submerged; otherwise similar to growth in tubes (Pl. 1, B). Differ markedly from other *Pythium* spp. in the combed-silk effect.

Corn-meal flasks.—Felted, compact white growth forming a dry mat about 2 or 3 mm. thick. Differs only in forming the more compact growth.

Oatmeal agar.—White aerial growth well defined after five days, loose and cottony, but more compact in central part of colony; after 14 days

becoming appressed and felted on upper half of slant, with more open texture toward base; drying down in three months to a uniformly compact white mat. No marked difference.

CARROT AGAR.—Growth prostrate, forming a thick gray sodden mat, wet-shining, irregularly wrinkled; aerial after three weeks, resulting in a uniform woolly white mass; later drying down to a prostrate deeply wrinkled dry mat bearing small floccose areas on upper part of slant, with loose cottony masses at base (Pl. 1, C.) Radiating irregular patches visible by transmitted light in central part of colony. Differs from other three species in absence of early aerial growth and in characteristic wrinkling.

STRING BEAN AGAR.—Prostrate dry mat deeply wrinkled in center; aerial growth after three weeks, forming a closely appressed felted mass, white at upper part of slant, becoming sepia brown toward center of slant, with loose cobwebby hyphæ at base; flattening down in three months to a thin dry mat, with marked wrinkling in center and the characteristic patchy appearance by transmitted light.

POTATO AGAR.—Differs from other three species in absence of early aerial growth and in wrinkling. Prostrate, smooth, sodden mat, bearing short downy hyphæ after 10 days, flattening down after three weeks to a gray, wet mat, slightly wrinkled at base of slant.

POTATO DEXTROSE AGAR.—Prostrate sodden mat, bearing tufts of loose to felty white hyphæ; deeply wrinkled in center after three weeks, light-grayish olive, with cobwebby tufts of aerial hyphæ scattered over lower half of slant; compact, felted tufts on upper half. Differs in presence of wrinkling from other three species.

SUGAR BEET AGAR.—Compact white aerial growth after one week, bearing olive-green slimy masses of oospores on center of slant; after two weeks, loose wooly-white hyphæ, partly overgrowing olive-green masses, later flattening down to a sodden, wrinkled, gray mat with scattered cobwebby hyphæ in lower part of tube. Characterized by presence of olive-green masses.

CONGO RED BEEF PEPTONE AGAR.—Very scanty surface growth, consisting of a few widely scattered prostrate hyphæ; surface of slant becoming dry and glossy; abundant submerged growth, filling the agar cylinder with long closely parallel hyphæ; very marked combed-silk effect and radiating patches by transmitted light; color of medium changed to deep Indian purple (*15*) within two weeks. No color change in others.

BEEF INFUSION PEPTONE AGAR.—Thin, sodden gray mat showing characteristic combed-silk effect and radiating areas; no aerial mycelium developed. Differs in having the combed-silk effect.

GERANIUM DECOCTION AGAR.—Growth similar to that on beef agar, except that the medium is discolored a greenish brown several millimeters in advance of growth. Growth much slower than that of other *Pythium* species.

STERILIZED GERANIUM STEM (with distilled water in tube).—Blackened in three days except part under water; scanty aerial growth on surface of stem; abundant diffuse growth out into the water. No marked difference.

POTATO CYLINDERS.—Thick, sodden gray mat, bearing a few small tufts of short downy hyphæ; slant covered after three weeks with compact downy to felty white mycelium, becoming loose and cottony at base of cylinder. No marked difference.

SUGAR-BEET CYLINDERS.—Felted white mass within two weeks, closely appressed, becoming cottony toward base; after two months forming a uniformly compact dry white mat. Differs in extreme compactness of growth from each of the others.

STRING BEAN PODS (with distilled water in tube).—Sodden gray mat covering pod, bearing scattered tufts of short downy hyphæ, which mat down after three weeks or persist in spots; thick gelatinous mass floating on the water in the tube, with many hyphæ diffusing underneath. No marked difference.

SOYKA RICE.—Abundant white cottony growth, becoming felty and compact after three weeks; no color change after three months. Differs in the more profuse growth from the three others.

VIABILITY

Duplicate tube cultures on various media, which had been kept at room temperatures (18° C. in winter to 29° in summer) for 6 to 16 months, were tested for viability in two experiments. A sterile decoction of Quaker oats was poured into each tube and removed after absorption of fluid by the dried agar. Melted corn-meal agar, cooled down to 38°, was then poured on the slants; the tubes were replaced in the incubator and examined up to three weeks. Transfers from cultures which showed growth on the fresh agar were made to oatmeal agar and carrot agar for identification and comparison with similar transfers from a stock culture. Results are given in Table I.

TABLE I.—*Viability experiments*

Medium	Date inoculated	Date tested	Time elapsed	Observations (duplicate tubes)
Oatmeal agar	Dec. 31, 1919	Aug. 25, 1920	7 months, 25 days	Alive in one tube.
Potato agar	Jan. 26, 1920	do	7 months	Alive in both tubes.
Do	Feb. 25, 1920	do	6 months	Do.
Oatmeal agar	Sept. 8, 1921	Aug. 22, 1921	11 months, 14 days	Dead in both tubes.
Corn-meal agar	do	do	do	Alive in one tube.
Congo Red agar	do	do	do	Dead in both tubes.
Beef agar	do	do	do	Do.
Potato agar	do	do	do	Do.
Potato dextrose agar	do	do	do	Do.
Carrot agar	do	do	do	Alive in both tubes.
Bean agar	do	do	do	Alive in one tube.
Steamed rice	Apr. 14, 1920	do	16 months, 8 days	Dead in both tubes.
Sterilized geranium stem	Apr. 17, 1920	do	16 months, 5 days	Do.

The experiments indicate viability after eleven and a half months at room temperature on carrot agar, bean agar, and corn-meal agar; and after seven and nearly eight months on potato agar and oatmeal agar, respectively. The fungus was not recovered after 11 to 16 months on seven other media used. Viability appears to be connected largely with the water-retaining power of the media. Death occurred much sooner on plate cultures, where the large surface and thin layer of medium leads to rapid desiccation. On October 8, 1923, sterile tap water was poured over corn-meal agar plate cultures 3 and 6 months old and bone dry. Absorption and softening soon took place, but no germination of the numerous oospores and sporangia was observed after three days, nor did growth occur on replacing the water with fresh cornmeal agar. Stock cultures in oatmeal agar tubes have, however, been kept in the icebox for 18 months without loss of viability.

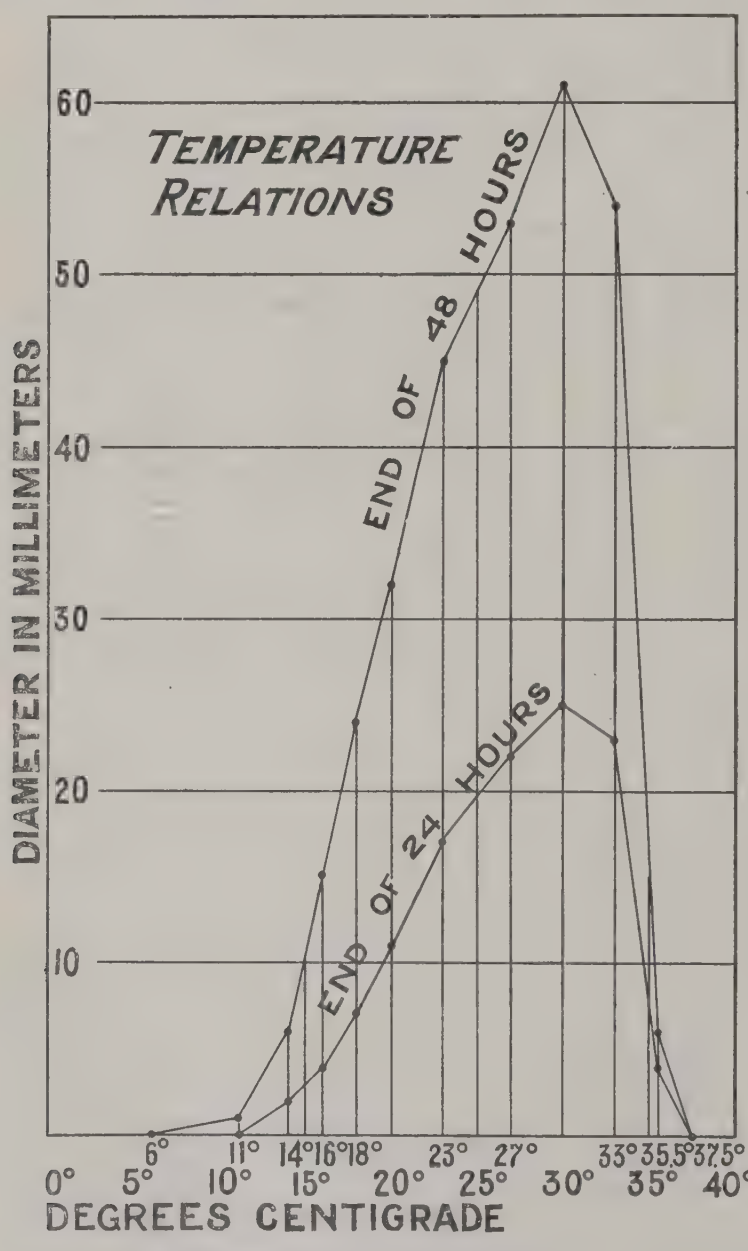


FIG. 3.—Graphs showing colony diameters at 2°–37.5° C. for 24 and 48 hours

TEMPERATURE RELATIONS

The Figure 3 outlines the growth curves averaged from two experiments with plates in ice thermostats and warm incubators, ranging from 2° to 37.5° C. Triplicate plates of corn-meal agar were placed in each compartment and measurements of the colony diameter were made at 24-hour intervals. Inoculations were made from a 3-day-old corn-meal agar plate, which was cut up into one-sixteenth inch

squares, each square then being planted in the center of the fresh plate. Before inoculation all plates were kept overnight in their respective compartments to avoid lag effects.

No growth takes place at 11° or at 37.5° within 48 hours or for any subsequent period at the latter temperature. Very slow growth is evident at 5° after 144 hours (3 mm., not shown in fig. 3). The optimum temperature centers around 30°. A marked difference in temperature ranges above and below the optimum may be observed, the growth rate falling away much more rapidly above the optimum temperature.

GENERAL DISCUSSION

TAXONOMY

The general characters of this fungus and the fact that the zoospores are not formed within the original sporangial wall, but within an evanescent vesicle containing the undifferentiated extruded contents of the sporangium, place this organism in the genus Pythium (Pringsheim). Preliminary complete differentiation before entrance into the vesicle has not been observed, although scores of sporangia have been germinated and studied; the only other method of germination noted was by germ tube, as described above for older sporangia.

The characters of the sporangium and oospore place this organism in Butler's subgenus Sphærosporangium (*6*), among the species with smooth-walled oospores lying free in the smooth oogonium (*P. de baryanum*, *P. ultimum*, and *P. vexans*). It differs from the first, which was studied at the same time, in: (*a*) Smaller oospores and oogonia; (*b*) finer mycelium; (*c*) very marked cultural differences; (d) inability to attack water cress, cucumbers, and radishes; (*e*) preponderance of zoosporangia rather than conidia; (*f*) the very characteristic antheridium. It differs from *P. ultimum* in being parasitic, in producing zoosporangia rather than conidia (which is a distinguishing character of *P. ultimum*), and in the shape of the antheridium.

In this last character it comes nearest to De Bary's *Pythium vexans*, which he characterized (*1*, *2*) by: (*a*) The peculiar insertion of the oogonium, which is sessile on the outside of the mycelium or inserted with a broad base into the mycelial tube; (*b*) the broad appressed antheridium fused to the oogonium, although his figure (*2*, *Pl. 5*, *fig. 3*) shows one clavate antheridium; (*c*) saprophytic habit; (*d*) no sporangia or conidia were observed. Butler (*6*) adds that the tapering of the hyphæ into very fine filaments distinguishes it from any other species he studied. He was more successful than De Bary in finding sporangia, which were "rare, scarcely ever spherical or oval, but irregularly pear-shaped, ovoid or subangular."

The organism described in this paper resembles *P. vexans* in the shape of the broad appressed antheridium, but differs in the following respects: (*a*) The oogonia are not inserted with a broad base, but are borne on a slender stalk (Pl. 5, B, C, D); (*b*) it is parasitic on Pelargonium and Coleus; (*c*) sporangia are formed abundantly on various media and are oval to spherical (Pl. 4, F), except when intercalary; (*d*) the hyphæ are cylindrical with rounded tips (Pl. 4, E) and do not taper to fine points. It is considered a distinct species, for which the name *Pythium complectens* n. sp. (referring to the clasping antheridium) is proposed. The technical description follows.

Pythium complectens, n. sp. Hyphæ coenocytic, hyaline, 1.70 μ to 4.85 μ; cylindrical with rounded tips, forming a strongly parallel silky growth on solid media; acid produced changing color of Congo Red agar; sporangia abundant, vacuolate, produced singly, spherical when terminal, oval to subspherical when intercalary, without papilla, average 21.8 μ in diameter, range 16.4 μ to 27.3 μ, germinating by extrusion of undifferentiated contents into vesicle in which zoospores are formed, not proliferating; zoospores broadly lenticular, with two cilia at hilum, with single vacuole, 10 to 26 formed per sporangium, 5.9 μ to 8.5 μ wide by 10.6 μ to 11.5 μ long, rounding up and germinating by a tube; oospores single, smooth walled, spherical, free in the oogonium, wall light yellow to sepia brown, abundant in host tissues, average 16.2 μ in diameter, range 11.3 μ to 20.8 μ; oogonia smooth, subspherical, borne on a slender stalk, average 18.6 μ in diameter, range 13.2 μ to 23.3 μ; antheridium single, one-celled, arising from adjacent hypha or below oogonial stalk, persistent, varying from a trumpet shape flaring out at region of attachment, to a broad irregularly lobed mass clasping a large part of the oogonium and fused with it. Parasitic on Coleus and Pelargonium cuttings, causing a black stem-rot; inducing a resistance reaction in the latter host characterized by the formation of a cork cambium, barring further progress of the hyphæ after infection has proceeded some distance from the point of inoculation.

FERTILIZATION AND MATURATION PHENOMENA

A striking feature of fertilization is the absence of a fertilization tube, coincident with the close contact of the contracted oosphere with some part of the oogonial wall immediately underneath the clasping antheridium. Butler (*6, p. 52*) found no fertilization tube in a species of Aphanomyces, in which the antheridium closely encircled the oogonium, but thought it possible that a nucleus was transferred before the oosphere receded from the oogonial wall. He figures a fertilization tube, however, in *P. vexans*, which has the same encircling type of antheridium, but no close contact of the fused wall and oosphere, so far as can be judged from his drawings. The close relation of oosphere, oogonial wall, and antheridium in the species here described is brought out clearly in photographs of living material just prior to or at the time of fertilization. (Pl. 4, *Aa, Af, Ba, Bb, Ca.*) Under these conditions the direct passage of the antheridial contents into the oosphere through a hole dissolved in the walls between is thoroughly compatible with the clasping nature of the antheridium, which insures sufficient adhesion to eliminate the necessity of the anchorage afforded by a fertilization tube in species having a narrow clavate antheridium. The fact that a lateral pressure is exerted by the antheridium is well shown by the depressed condition of the oogonial wall below it, displacing the otherwise spherical shape of the oogonium (Pls. 4, 5, all oospore figures).

The inception of the exospore wall immediately upon fertilization and rounding up of the oosphere has not been described in detail in this genus so far as the writer is aware; most authors record the appearance of the complete membrane without figuring possible intermediate stages. Trow (*22*) states that "the egg rounds itself off and appears covered with a membrane before the last traces of protoplasm leaves the antheridium." Miyake, working with *P. de baryanum* (*13*), uses similar terms: "Soon after the discharge of the antheridial contents into the oosphere a thin membrane is formed around the latter. This is the beginning of the exospore.' The figures of both authors show a completely encircling membrane of appreciable thickness, immediately following the highly contracted, smooth-outlined oosphere. De Bary noted a thin hyaline membrane around the oosphere before fertilization in *P. de baryanum;* its first figured appearance is a line almost completely encircling the still rough oosphere (*3, Pl. 1, fig. 3, 4*). Ward (*23*), working with the same species, states that "meanwhile [during fertilization] a very delicate skin had been formed over the now smooth exterior of the oosphere," but his figures do not show how it was formed. The general impression left by these authors is of a simultaneous appearance of a thin hyaline membrane over the entire oosphere. This was not found to be the case in the species here studied, in which the membrane is first visible just within the smoothed periphery as a short, narrow hyaline strip (really a disk in terms of three dimensions) which gradually extends tangentially around the periphery until the entire oosphere is clothed with a narrow, hyaline membrane (Pl. 5, *C, d, e, f*).

SIGNIFICANCE OF THE PYTHIUM TYPE OF SPORANGIAL GERMINATION

The normal process of sporangial germination in this genus results in the transformation of the entire sporangial contents into zoospores and their facile escape through the very delicate vesicle wall without any waste of zoospore-forming material or inherent obstacles to dissemination when once formed. It was frequently observed, however, that the undifferentiated mass within the vesicle sometimes moved off (as if the bladder had been disrupted prematurely), failed to differentiate, and degenerated into a mass of slime and globules. This was frequently correlated with impacts by freshly formed zoospores, singly or in groups, from near-by sporangia, and must unquestionably occur in nature through mechanical injury by the abundant motile microscopic fauna and flora of the soil. Another abnormal condition, observed in only two cases, was the disjunction of the protoplasm during its passage through the tube; most of it entered the vesicle and underwent the usual process, while the part broken off and left behind in the sporangium failed to emerge, but formed a few normal zoospores which remained entrapped and finally degenerated (Pl. 4, H). The rarity of this condition in Pythium is indicated indirectly in that incomplete exit of the sporangial contents, resulting in entrapped zoospores, is neither mentioned nor figured in Butler's monograph (*6*) nor in Ward's detailed accounts (*23*).

Instances of imprisoned zoospores are, however, not uncommon in allied genera in which they are completely

differentiated before emergence. Rosenbaum, working with Phytophthora (*16*), states that "frequently for some reason a few of the swarmspores do not emerge with the majority." In forms in which the vesicle occurs (accompanied by preliminary differentiation in the original sporangium), he finds that "after the liberation of the swarmspores, the vesicles begin to contract, all signs of the opening disappear, and if any zoospores remain they are unable to escape." Coker, in his recent monograph of the Saprolegniaceae (*7*), notes that "in both Saprolegnia and Achlya it frequently happens that the discharge of the spores is only partial, a few, or even a good many spores being left in the sporangium. These retained spores may emerge from their cysts as normally, for a second swimming stage, moving about within the sporangium until they find their way out by its mouth, if they ever do."

Correlation of these observations indicates that the Pythium type of zoospore formation *per se* is by far the better mechanism for securing maximum dissemination with minimum loss of spore-forming material for the following reasons:

(1) The migrating protoplasmic mass, covered as it is by a single plasma membrane, is kept together by cohesion and surface tension, both of which are powerful enough (except in rare and clearly pathological cases) to force the entire mass out as a whole, once emergence has been initiated by the propulsive force engendered by imbibition and swelling of a colloidal aggregate in the presence of an available opening. The operation of these factors is clearly evident to anyone watching the pulling together of the migrating mass when half way through the tube, and its subsequent rounding up when wholly within the elastic vesicle. Compared with other genera, it is in sharp contrast with the emergence singly of a collection of fully formed zoospores, the vanguard of which must be subjected to the propulsive force of swelling, which is necessarily exhausted when the total potential swelling of the remaining individuals is equal to the volume of the sporangium; the exit of the remainder, as De Bary (*4*), Coker (*7*), and others have shown, is largely dependent on their connection with the preceding spores by delicate protoplasmic threads and ciliary entanglements rather than by their own unaided efforts. Considering the fragility of these connections and the miniature turmoil at the time of emergence, it is not surprising that zoospores are often left behind and fail to escape, as the above-quoted authors have observed. In the Pythium type of egress, however, the entire sporangial contents are removed *en masse* and under one enclosing membrane, thereby incurring a minimum risk of wasting spore material by imprisonment in the sporangial walls.

(2) Provision for maximum facility of dissemination, once the zoospores are formed, is secured in Pythium by the presence of an extremely tenuous, fragile membrane around the zoospore mass, capable of disruption with the greatest ease and thereby enabling all zoospores formed to swim away, as contrasted with the rigid walls surrounding the zoospores in other genera.

These conditions and their results—emergence *en masse* and fragility of the vesicle membrane—point clearly to the above conclusion as to the greater intrinsic efficiency of the Pythium type of sporangial germination, and to the interpretation that this type constitutes a definite adaptation securing minimum waste of spore-forming material combined with maximum ease of dissemination. That it did not become the prevailing type in zoospore-forming fungi can be traced to another factor, the clue to which is afforded in the above-recorded observation that the delicate vesicle containing the undifferentiated mass was susceptible to impacts leading to mechanical injury and degeneration before zoospores could be formed. It is clear that maximum dissemination is here obtained at the expense of protection at a critical stage, such as is afforded in genera in which the zoospores are formed within the rigid sporangium. The great advantage of the latter method lies in the fact that some at least of the swarmspores are sure to escape and perpetuate the race; in the Pythium type, the whole output of the sporangium is lost if the undifferentiated mass is mechanically injured in its exposed position. Evolutionary tendencies in matters of reproduction in higher plants and animals are in the direction of specialized and well-protected off-spring in small numbers rather than along the lines of quantity dissemination of the unprotected many; it is only necessary to consider this to see that a specialized adaptation which secures the latter end, such as we have in Pythium—admirable a mechanism as it intrinsically is—yet insufficiently proof against unfavorable conditions, can not imprint itself permanently on derived genera. Hence, in the closely allied and probably derived Phy-

tophthora, the tendency to the well-protected method of differentiation within the original sporangium finds full expression; the vesicle present in some species no longer serves to hold the undifferentiated spore mass but remains as a functionless inheritance from Pythium like ancestors, finally disappearing in other members of the genus.

RESISTANCE PHENOMENA

The formation of a protective cork cambium tending to inhibit the progress of infection, such as occurs in this disease, has been noted elsewhere by various workers. Lutman (*12*) interpreted potato scab as a successive series of cork layers laid down in advance of the parasite, which, however, was apparently powerful enough to pass through each layer and stimulate the production of another layer deeper in the tissues. He noted the disappearance of starch from healthy cells in the vicinity of the cork cambium, a condition which is also present in this geranium disease. Dufrenoy (*8*) reports protective cork formation in a chestnut disease. Tisdale, working with flax wilt (*21*), noted suberization of groups of cells adjacent to those attacked by *Fusarium lini*, and pointed out the necessarily chemical nature of the initial interacting forces involved. Protective cork formation accompanied by disappearance of starch from adjacent healthy cells is figured by Erwin F. Smith in a potato tuber rot caused by *Bacillus caratovorus* (*19, fig. 174*).

The effectiveness of suberized cell walls in preventing the progress of infection has been emphasized by Shapovalov and Edson (*17*) in their work on wound cork formation. In the case of Pythium it is in accord with the mechanical nature of hyphal penetration as interpreted by Hawkins and Harvey (*9*), although the high resistance of suberized walls to solution by a large number of reagents should not be overlooked. The inability to obtain infection on uninjured geranium stems with the organism here reported, and the low amount of infection on cuttings made two days before inoculation, are clearly correlated with difficulty in penetrating cork layers, which, as related to the former case, are often 10 cells thick beneath the cuticularized epidermis.

It is of interest to note that the organism at present under consideration produces a diffusible substance, evidently an acid, capable of changing the color of Congo Red agar to a deep India purple (*vide* Cultural studies), whereas the other three *Pythium* spp. studied at the same time (to be reported on in a later paper) caused no color change in this medium; nor did the latter ever induce cork formation in inoculated plants, which usually succumbed completely. While the nature of the diffusible substance produced by the cork-inducing Pythium has not been determined, a possible cause and effect relation is clearly indicated in the correlation between its production and the presence or absence of a resistance reaction evidenced by the stimulation of cell division to form a cork cambium. Stimulation of cell division by diffusible substances produced by a parasite is considered by Kunkel (*10*) as a possibility in the case of the Plasmodiophora disease of cabbage; further evidence is available in the numerous experiments of Erwin F. Smith with *Bact. mori*, *Bact. solanacearum* (*19*) and particularly with known by-products of *Bact. tumefaciens* (*18, 20*).

SUMMARY

1. A stemrot of geranium (Pelargonium) cuttings caused by *Pythium complectens* n. sp. is here described.

2. The disease consists of a progressive basal blackening accompanied by pectinization and soft rot of pith and cortex. Infection stops at a sharp line of demarcation 20 to 40 mm. from the base within six to eight days after inoculation.

3. Stoppage of infection is due to a host resistance reaction manifested by the formation of a cork cambium completely across and within the stem, barring further progress of the hyphæ after infection has already proceeded some distance from the point of inoculation. It is accompanied by the disappearance of starch from healthy cells in the vicinity of the cambium.

4. This reaction is specific for this particular host and organism, and was not found in the case of three other *Pythium* spp. studied which caused complete destruction, nor in the case of Coleus cuttings infected and completely rotted by this organism.

5. Characteristic signs of the disease can be caused with pure culture inoculations of this organism on any wounded part of the stem. Reisolation, reinoculation, and constant presence of characteristic oospores in undecayed parts of diseased tissues have established pathogenicity. Coleus cuttings are susceptible, but not cucumber, radish, or cress seedlings.

6. The hyphæ of this fungus are hyaline, coenocytic, and cylindrical

with rounded tips. Sporangia are abundant in culture media, and are regularly oval to spherical. Germination is by extrusion of the undifferentiated contents through a short tube into an evanescent vesicle in which the zoospores are differentiated; germination by tube takes place in older sporangia. Proliferation has not been observed.

7. The oospores are smooth walled and lie free within the smooth oogonia. The latter are borne at the tips of slender branches. The antheridia are particularly characteristic, varying from a trumpet form to a broad, irregularly lobed mass clasping and fused with a large part of the oogonial surface.

8. Fertilization takes place by direct passage of the antheridial contents through a hole in the fused oogonial and antheridial walls into the contracted and immediately subjacent oosphere. A fertilization tube has not been observed.

9. Formation of the exospore wall is initiated by the peripheral spread of a narrow, clear band (a disk in 3 dimensions) within and around the smooth outlined oosphere, completely enclosing it.

10. Viability and cultural characters on 16 media are given in detail.

11. Optimum growth takes place at 30° C. The maximum temperature is 35.5°; the minimum 5° (144 hours).

12. Comparison of the Pythium type of zoospore formation with that in other genera points (*a*) to the greater intrinsic efficiency of the former type in view of the advantages herein discussed resulting from emergence of undifferentiated contents *en masse*, and the fragility of the vesicle membrane; (*b*) to an interpretation of this type as an adaptation which secures minimum waste of spore-forming material combined with maximum ease of dissemination, at the expense, however, of protection at a critical stage. That it did not become fixed in derived genera is an expression of the general evolutionary tendency toward protection of offspring rather than facility in dissemination.

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ALTERNARIA LEAFSPOT AND BROWNROT OF CAULIFLOWER [1]

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INTRODUCTION

In 1918 the writer's attention was called to a disease of cauliflower [2] which was causing considerable damage during transportation to the market. Inspectors for the Bureau of Agricultural Economics reported it frequently from the Chicago and New York markets on cauliflower from California and the Northwest. The disease probably developed in transit, since heads said to be healthy when placed in the cars were badly diseased on reaching their destination. From 10 days to 2 weeks usually elapse between the cutting of the cauliflower on the Pacific coast and its arrival in the eastern markets. This probably affords ample time for the disease to develop. However, there was no information available regarding the cause of this disease, its origin, or the conditions under which it developed. The investigations discussed in this paper were undertaken with a view to furnishing such information.

HOSTS

Although the brownrot as such occurs on only the heads of cauliflower, a leafspot caused by the same fungus occurs on a number of cruciferous plants. The leafspot on cabbage is especially well known. The writer has been able to obtain infection of young seedlings of kohlrabi, Brussels sprouts, broccoli, cabbage, and cauliflower in the greenhouse. An attempt to infect white mustard seedlings failed. A number of other closely related plants are also hosts for this disease.

NAME OF THE DISEASE

The leafspot is usually referred to as "Alternaria" leafspot but is sometimes called "black" leafspot and "blackmold" The term "brownrot" has been applied for the past several years to certain brownish-colored lesions of unknown origin commonly found on the curds of cauliflower on the market. Although these lesions do not all have a common origin, it is thought that the disease here discussed is their most common cause. The term "brownrot" is fairly descriptive of the disease, and since it is already in use by tradesmen it will be retained here. But care should be taken not to confuse this disease with other rots of the curd of this host which may also have a brown appearance. One such rot which is especially prevalent and which is most likely to be confused with the disease under consideration is of bacterial origin and is known by the trade as "slimy softrot." The term "brownrot" was applied in the past to a disease caused by *Bacterium campestre* (Pammel) Erw. Sm., but the name "black rot" is now used exclusively to designate this trouble.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Alternaria leafspot has a very wide distribution, both here and abroad. The disease on the curd of cauliflower is probably coextensive with the crop but is more destructive when the host is shipped long distances or is held for a considerable period under conditions favorable for its development. Puttemans (*13*)[3] in 1911 described what no doubt was this same disease occurring on curds of cauliflower found on the markets of Rio de Janeiro. He studied the disease somewhat and decided that it was caused by *Alternaria brassicae* (Berk.) Sacc. The disease was found to develop in transit during warm moist weather, notwithstanding the fact that the time consumed in packing and shipping the cauliflower was not longer than three to four days. The writer's data show that this rapid development of the disease may be expected at high temperatures and humidities.

[1] Received for publication April 10, 1924—issued January, 1925.

[2] The term "cauliflower" as here used also includes broccoli.

[3] Reference is made by number (italic) to "Literature cited," pp. 441-442

Fawcett (*8*) states that *A. brassicae* causes much damage to cabbage in Florida some seasons. Higgins (*11*) likewise reports this disease as having been very destructive on collards in Georgia for several years. Harter and Jones (*9*) state that black leafspot causes considerable damage to cabbage and collards in this country and in Europe. Chupp (*3*) records the fact that on Long Island in the cabbage-seed growing district Alternaria always does injury as a seed-pod spot. The literature, both domestic and foreign, is replete with references to Alternaria leafspot on cabbage and other cruciferous hosts. Notwithstanding its prevalence, the disease is usually of minor importance on cabbage in the field, since it ordinarly occurs only on parts of the host which have become low in vitality. With the exception of that by Puttemans (*13*), no reference to the occurrence of this disease on the curd of cauliflower has been found. No doubt the brownrot has been prevalent for a long time without having received much attention from scientists, due in part at least to the fact that until recent years little time has been given to the study of transit diseases. As stated above, the disease of the curds seems to be most prevalent on cauliflower from the Pacific coast. This does not mean that it is necessarily more common or destructive there. The writer has sought many times for this disease on the heads of cauliflower in the vegetable-growing section about San Francisco during the season of 1922–23 but never found it except on heads that were overmature or that had been cut from the plants and left lying on the ground. The growers in the Los Angeles section claim that this disease appears during the latter part of their shipping season. While the leafspot stage was not common in the San Francisco section it was very prevalent about Los Angeles during the season of 1922–23. Probably cauliflower from no other region is subjected to conditions so favorable for the development of the disease as is that from the Pacific coast during the period of transit across the continent to the eastern markets. Nevertheless, the writer has seen the typical brownrot lesions on cauliflower from New York. Badly diseased heads have also been obtained from the Washington, D. C., city market. Some of these were locally grown while others were said to have come from Florida. There is little doubt that the brownrot may be found wherever cauliflower is grown, provided it has been subjected to proper environmental conditions. But it appears to be of little importance except as a transit trouble.

A few figures from the reports of the Bureau of Agricultural Economics indicate some of the losses caused by this disease in transit (Table I).

Except under conditions of high humidity, the disease develops slowly and causes greater loss by badly disfiguring the leaves and heads than by actually destroying them. Under field conditions the leaves may be so badly affected that the vitality of the plants is lowered and the size of the heads consequently reduced. Other microorganisms such as soft-rot bacteria and species of Fusarium and Rhizopus also enter the lesions and initiate other types of decay.

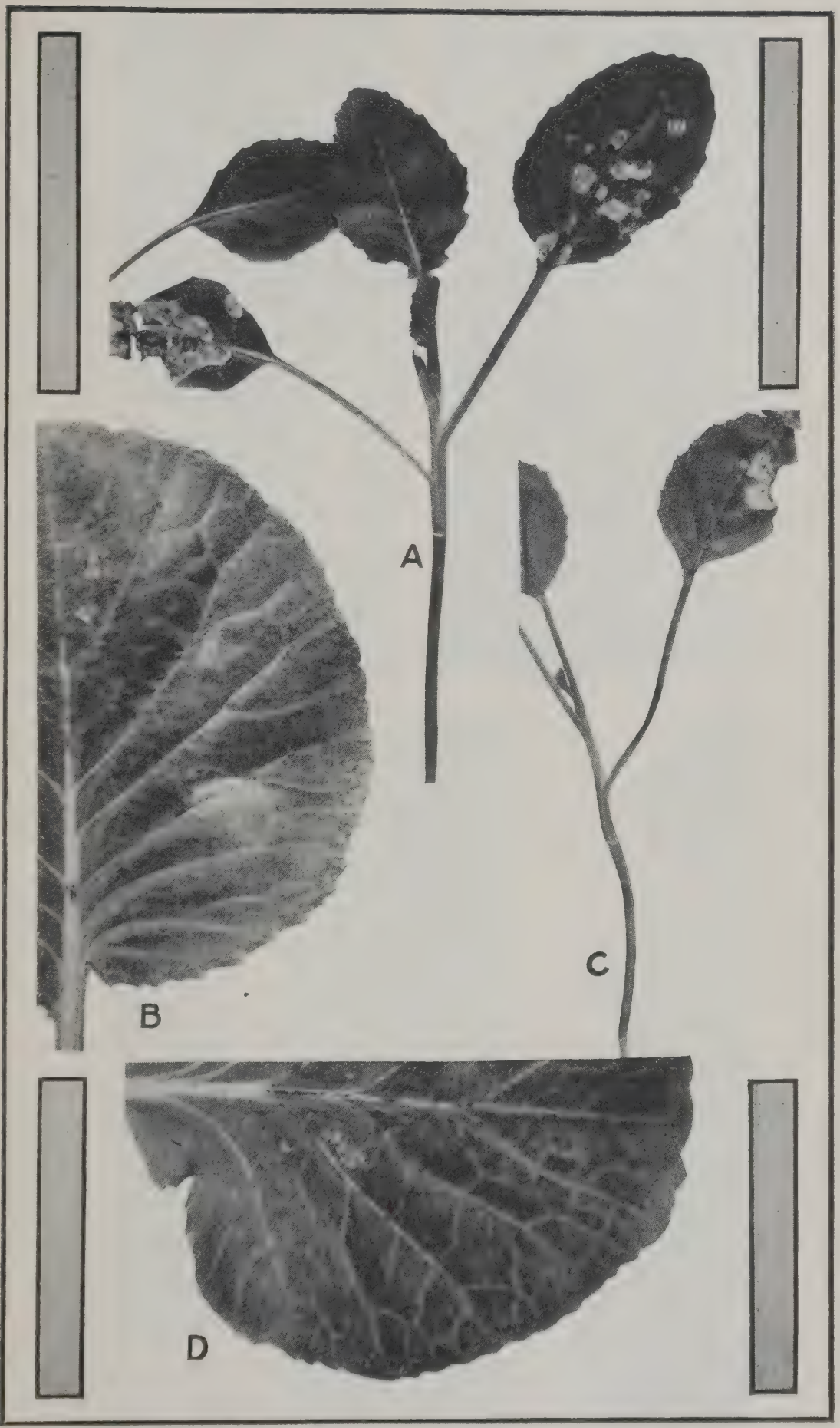
SYMPTOMS OF THE DISEASE

In the writer's experiments the disease first became evident on the leaves, petioles, and stems of young plants as small dark brown to almost black spots about 1 mm. in diameter. The ultimate size and character of the lesions depended to some extent on the humidity and on the nature of the tissue affected. Three types of lesions usually developed on leaves of cauliflower inoculated in the greenhouse (Pl. 1 and 2). On the lamina the spots remained small (about 1 mm.) and dark brown, developing but little after the first three days (Pls. 2, *b*), or they enlarged, forming lesions somewhat circular in outline from 0.5 to 1 cm. or more in diameter and grayish to brown in color. Scattered promiscuously about the center of these spots were minute black areas where the fungus was fruiting. These spots sometimes coalesced, forming large irregular areas, often involving half or more of the leaf (Pl. 1, A, C; Pl. 2, A, B, C, D). The diseased tissue shaded off from brownish to yellow and then to the normal green of healthy

EXPLANATORY LEGEND FOR PLATE 1

A and C.—Early Snowball cauliflower seedlings grown in the greenhouse, showing the type of lesions produced by *Alternaria brassicae* on the leaves and petioles. Photographed 3 days after inoculation. Natural size

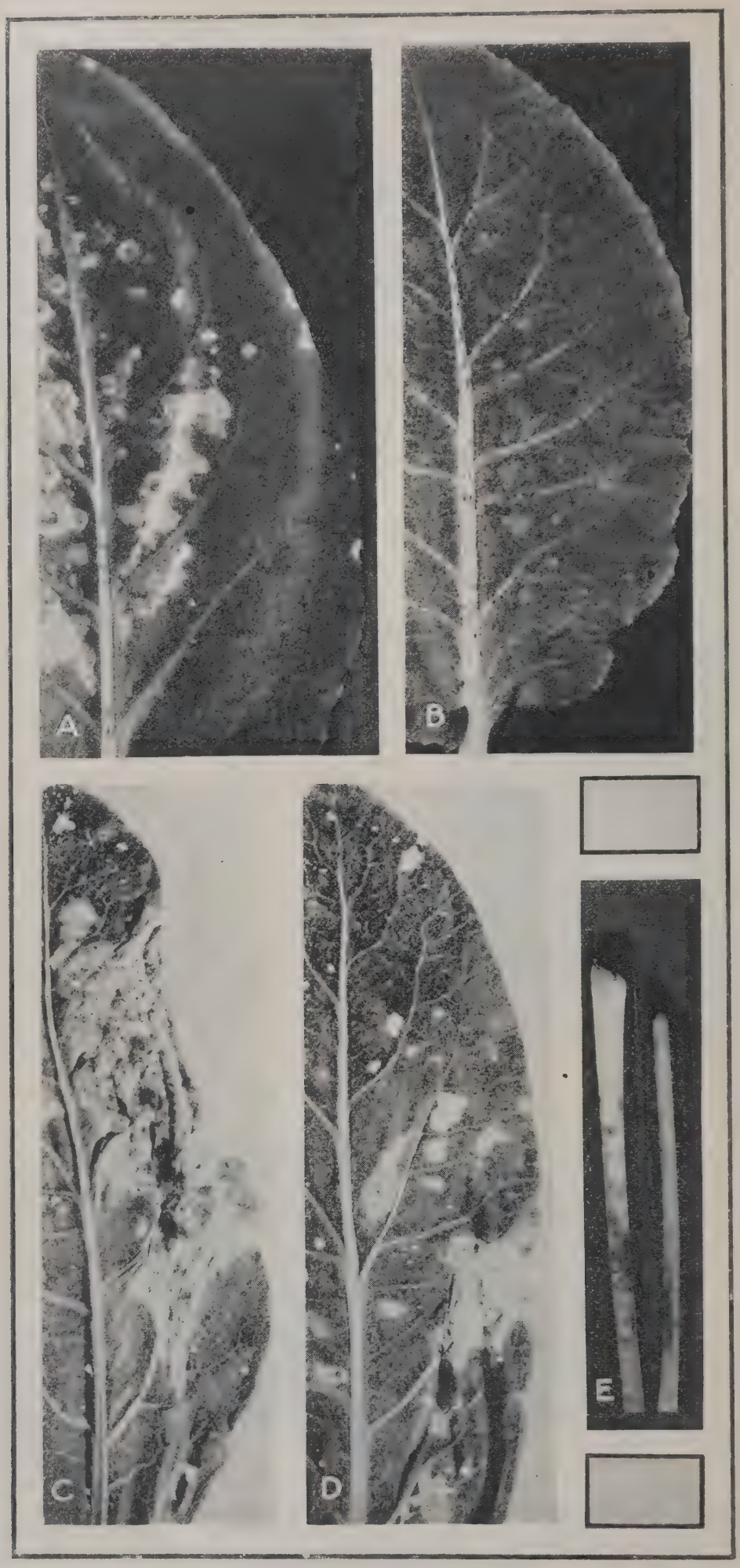
B and D.—Seedling cabbage leaves grown in the greenhouse, showing the circular to irregular, light-colored lesions caused by *A. brassicae* as well as the small, dark-colored ones, the latter being limited largely to the veins. About natural size



Alternaria Leafspot and Brownrot of Cauliflower

Plate 1

(For explanatory legend see p. 422)



Alternaria Leafspot and Brownrot of Cauliflower Plate 2

(For explanatory legend see p. 425)

tissue. Lesions of the third type resembled those of the first, except that they appeared on the larger veins, petioles, and stalks, and were linear in shape, having the longer diameter parallel to the veins, petioles, or stems. Under conditions of high humidity these lesions sometimes developed rapidly, so that the parts attacked were soon decayed through. Badly affected leaves finally turned yellow and dropped off. The area of leaf tissue which might have functioned in photosynthesis was greatly reduced and the plants failed to develop properly. Stems of seedlings were frequently weakened by the cankers produced by this disease, so that they broke over and died. Under conditions of high humidity the dead areas of the leaves were covered with hyphae and spores which gave them an olivaceous color.

In cauliflower leaves inoculated in the field (Pl. 2, C, D), large areas of leaf tissues were killed. This dead tissue was papery in texture and bore black spore masses. The high humidity and temperature at the time of inoculation and for several days thereafter no doubt account for the severity of the infection. The symptoms of the disease on cabbage leaves are for the most part the same as those on cauliflower. Plate 1, B and D, shows the different types of lesions on young cabbage leaves inoculated in the greenhouse.

This disease causes a browning of the individual buds or groups of buds of the curd. Plate 3, B, shows a very early stage of the disease on the curds, resulting from 5-day-old artificial inoculation. The color of the affected tissues varies with age as well as with the conditions of temperature and humidity. The infected blossoms are at first light brown, darkening with age to nearly chestnut brown, which is usually followed by an olivaceous color, due to the development of dark-colored aerial hyphae and spores. Plate 3, C, shows the same head illustrated in Plate 3, B, photographed 13 days after inoculation. The diseased spots had enlarged considerably and were of the typical olivaceous color. A similar condition is shown in Plate 3, D, which is a photograph of a naturally infected cauliflower head obtained from the market. Plate 4 shows a head which was inoculated in the field. Its flower stalks are elongated preparatory to the formation of seed. The blackened tissue was covered with spores and hyphae.

Lesions caused by this disease may be easily confused, especially in their early stages, with bruises and with soft-rot produced by bacteria. Microscopical examination is often necessary to determine the true nature of some of the small lesions. As the decay induced by bacteria develops, the tissue becomes soft, wet, and usually slimy. The tissue rotted by bacteria is light brown and has a water-soaked appearance.

ETIOLOGY OF THE DISEASE

In 1918, when this work was begun, the etiology of the disease on the curd of cauliflower was unknown, at least as far as could be ascertained from the literature. Isolations were made from a large number of affected curds of cauliflower from as many sources as possible and a pure culture of a species of Alternaria was almost always obtained. Careful study of this fungus has shown it to be the same as the one that causes the leafspot of cabbage and related plants, namely, *Alternaria brassicae* (Berk.) Sacc. This fungus was first described by Berkeley as *Macrosporium brassicae* (*1, p. 339*), but was later placed in the genus Alternaria by Saccardo (*14, p. 546*).

PATHOGENICITY

Although from brownrot lesions a single species of Alternaria was almost invariably obtained, sometimes a species of Fusarium, bacteria, or other species of Alternaria appeared in the plates. Inoculation experiments showed, however, that the species of Alternaria so commonly present in the lesions was the causal organism.

The method used in all of the experiments conducted to prove the pathogenicity of the strain of Alternaria isolated from the typical brownrot lesions was as follows: The preliminary tests were made in the greenhouse. Plants of different ages were sprayed

EXPLANATORY LEGEND FOR PLATE 2

A, B, and E.—Cauliflower leaves and petioles from greenhouse-grown plants, inoculated with *Alternaria brassicae* showing the circular, light-colored lesions which often coalesce, killing large areas of the leaf and also the small, dark spots (leaf B), which may spread but little. These leaves were sprayed with the same spore suspension at the same time and were held under identical conditions after being inoculated. Photographed 3 days after inoculation. *Alternaria brassicae* was recovered from both types of lesions. About three-fourths natural size

D.—Leaves of a mature field-grown cauliflower plant sprayed with a suspension of spores of *A. brassicae* during a hot, rainy period. The large, light-colored areas of the leaves were killed by the fungus. About one-half natural size. Photographed 6 days after inoculation

with a suspension of spores in sterile distilled water and then placed in a large moist chamber for about 48 hours. The leaves were first rubbed gently to remove the waxy bloom so that the spore suspension would stick. Likewise, the heads of cabbage and the curds of cauliflower were sprayed with a suspension of spores and then placed in moist chambers, usually battery jars lined with wet filter paper, and incubated at different temperatures. Heads and leaves of cabbage and cauliflower growing in the field were sprayed with a spore suspension and then the outer leaves were pulled up and tied about the head. Controls which consisted of plants from the same lot, treated in every way the same as those inoculated except that spores were not permitted to come in contact with them, were held in each experiment.

Usually isolations were made from the lesions formed on the inoculated host parts. In some instances a microscopical study was made which showed the typical Alternaria spores to be present in great abundance on the lesions. The organism isolated was shown to be capable of infecting a healthy host and producing the typical symptoms.

The results of several of the inoculation experiments are tabulated in Table II. A high percentage of infection was obtained in all cases. The number of spots produced on the heads and leaves varied from a few to very many, depending largely on the humidity and on the concentration of the spore suspension used. For the most part the leaves seemed more susceptible than the heads under field conditions, although a large number of infections on the latter were obtained. No injuries of any kind were made intentionally or could be observed on the curds of the cauliflowers before inoculation.

The experiments listed in Table II, together with those given in connection with temperature studies later, show conclusively that the species of Alternaria commonly associated with the leafspot and brownrot lesions is capable of producing such lesions under the conditions existing in these experiments. Other species of Alternaria were frequently found growing saprophytically on decaying cauliflower and cabbage. The fungi numbered 5089, 5095, and 4866a in Table II proved to be of this nature. Not only did these prove to be nonparasitic but they differed morphologically from the parasitic forms, all of which were alike.

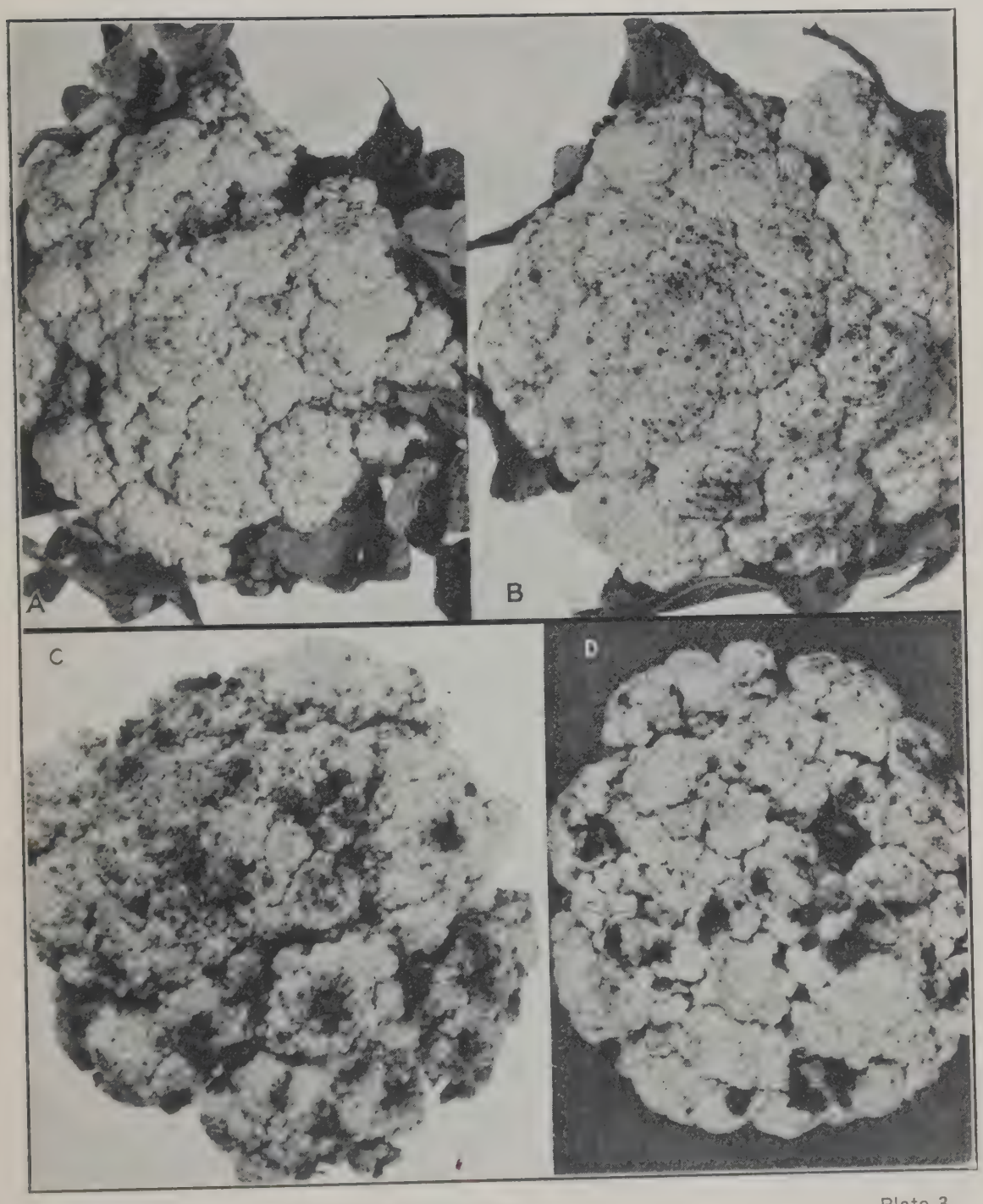
On the plants in the greenhouse which were inoculated on February 22, 1922, infection was evident as very minute dark-brown spots just visible to the unaided eye in 48 hours, when they were removed from the moist chamber. These spots were so numerous on some leaves that two-thirds or more of the entire leaf surface was involved. When examined 24 hours later, the large greenish water-soaked type of lesion was present and large areas of the leaves were killed. A similar condition is illustrated in Plate 2, C. The dead tissues later turned light brown in color and had a papery texture. Five days after the inoculations were made some of the petioles were so badly decayed that they broke off. Likewise, the laminae of some of the leaves were so severely affected that the leaves dropped off. So large a number of infections probably seldom, if ever, occurs on a host under natural conditions, yet one-half to two-thirds of the laminae of several of the leaves of cauliflower growing in the field which were inoculated on June 18, 1921, were dead six days later. Also the curds of these plants had large brownrot lesions at that time.

INFLUENCE OF TEMPERATURE ON SPORE GERMINATION AND MYCELIAL GROWTH

It has been pointed out that brownrot causes considerable damage to cauliflower while in transit. Cauliflower reported to be free from disease when placed in the car for shipment has often been found badly spotted on reaching its destination. The question naturally arises whether or not the fungus can develop at temperatures existing in the refrigerator cars in which cauliflowers are shipped. In order to determine this point, the effect of temperature on the rate of germination of the spores and the subsequent growth of the mycelium was studied. The method used was essentially the same as that described by Weimer and Harter (*18*). Preliminary tests showed that the spores germinated better in sweet-potato decoction (500 gm. sweet potatoes per liter) than in either tap or distilled water. Hence this medium was used.

EXPLANATORY LEGEND FOR PLATE 3

A.—Healthy head of cauliflower held as control
B.—Cauliflower head showing small brownrot lesions resulting from inoculation by spraying with a suspension of *A. brassicae* spores from a pure culture. Photographed 5 days after inoculation. Natural size
C.—Same cauliflower head photographed 13 days after inoculation. Natural size
D.—Cauliflower head showing natural infection with *A. brassicae*. Lesions appear as black spots



Alternaria Leafspot and Brownrot of Cauliflower

Plate 3

(For explanatory legend see p. 426)

Alternaria Leafspot and Brownrot of Cauliflower

Plate 4

(For explanatory legend see p. 429)

Loops of a spore suspension of the desired concentration were placed upon clean cover slips, which were then inverted over glass rings cemented to slides with a mixture of beeswax and vaseline, the cover slips being sealed to the rings with vaseline. A small quantity of the same medium used in preparing the spore suspension was placed in the bottom of each cell. The hanging drops were placed in the electrically heated and controlled Altman incubators as soon as possible after they were prepared. The temperature of these incubators remained quite constant, varying for the most part less than a degree, except for the lower ones, which depended upon the amount of ice in the cooling chamber, where there was sometimes a variation of 2° to 2.5° C. A pan of water was kept in the bottom of each chamber to keep the air moist. From four to eight hanging drops were used at each temperature. These were removed from the incubators at frequent intervals and observed under the microscope. The mounts were examined near the incubators, and were kept out usually less than a minute. Only at the highest temperatures was there any appreciable fluctuation due to the opening of the doors, and in these cases the readjustment occurred very quickly. In the different trials the average time found necessary for the spores which germinated first to produce germ tubes equal in length to the width of the spore was used as the time required for germination. The figures thus obtained, together with the average of the temperatures to which the spores were exposed in the different experiments, were used in plotting a curve which shows the variation in the time for germination to start due to the difference in temperature (fig. 1).

Other criteria might have been used for determining the influence of temperature upon this vital phenomenon. However, the important thing in connection with the study of brownrot is to know how soon infection can take place at the different temperatures and this obviously depends primarily upon the time necessary for the spores to germinate. In fact, it is usually of little practical importance to know whether or not all the spores germinate within a certain time at a given temperature since most fungi produce spores so profusely that if only a small percentage of them germinate a large amount of damage may result. About 99 per cent of the spores studied in these investigations germinated, except near the maximum temperature, where some of them were no doubt killed before germination started.

In the curve shown in Figure 1 the time in hours necessary for germination to take place is plotted on the abscissa, while the temperature in degress centigrade is plotted on the ordinate. The hanging drops were kept under observation for some time after germination started, to make sure that the other spores germinated soon afterwards. No germination took place at the maximum temperature tried (46° C.). As no temperature between 40.5° and 46° was tried, the temperature at which germination would have just taken place was not determined.

An examination of the curve shows that the optimum temperature for spore germination is somewhere between 33° and 35° C. From the optimum the time necessary for germination to begin gradually increased as the temperature became lower, but increased quite rapidly above this temperature until the maximum was reached. The minimum for germination was not determined accurately for want of a sufficiently low temperature. Germination took place in 48 hours at an average temperature of 1.5°. It is apparent from these data that so far as spore germination is a factor, infection by the brownrot fungus can take place in transit.

The effect of temperature upon the growth of the mycelium was studied in Petri dish cultures containing 20 cc. of a 2 per cent Irish potato agar. A small drop of a suspension of the spores in water was placed in the center of each plate with a 2 mm. platinum loop. The plates were placed in the incubators as soon as they were inoculated and the rate of growth determined by measuring twice daily the diameter of the mycelial disks formed. Five Petri dishes were used for each temperature. The experiment was repeated and the average diameters of the mycelial colonies as well as the average temperatures for the times that the cultures were grown were used in plotting the graphs in Figure 2. The base line of these

EXPLANATORY LEGEND FOR PLATE 4

Cauliflower head grown in the field, badly affected with brownrot resulting from artificial inoculation. This head has spread and the flower stalks have begun to elongate preparatory to blossoming. All of the blackened area was affected. About natural size

99186—25†——2

graphs shows the temperature in degrees centigrade; the perpendicular shows the diameter of the mycelial disks. The first graph shows the diameter of the mycelial disks at different temperatures at the end of 2 days, while the other graphs show their comparative diameters after 4, 6, 8, 10, and 15 days, respectively.

same throughout the experiment. No growth took place at 30.5° during the first two days, although a fair growth was made at 30°. There was some visible growth in four days at 33.5°, but none until the eighth day at the maximum temperature, at which a visible growth took place (36°). There was no growth even after 15

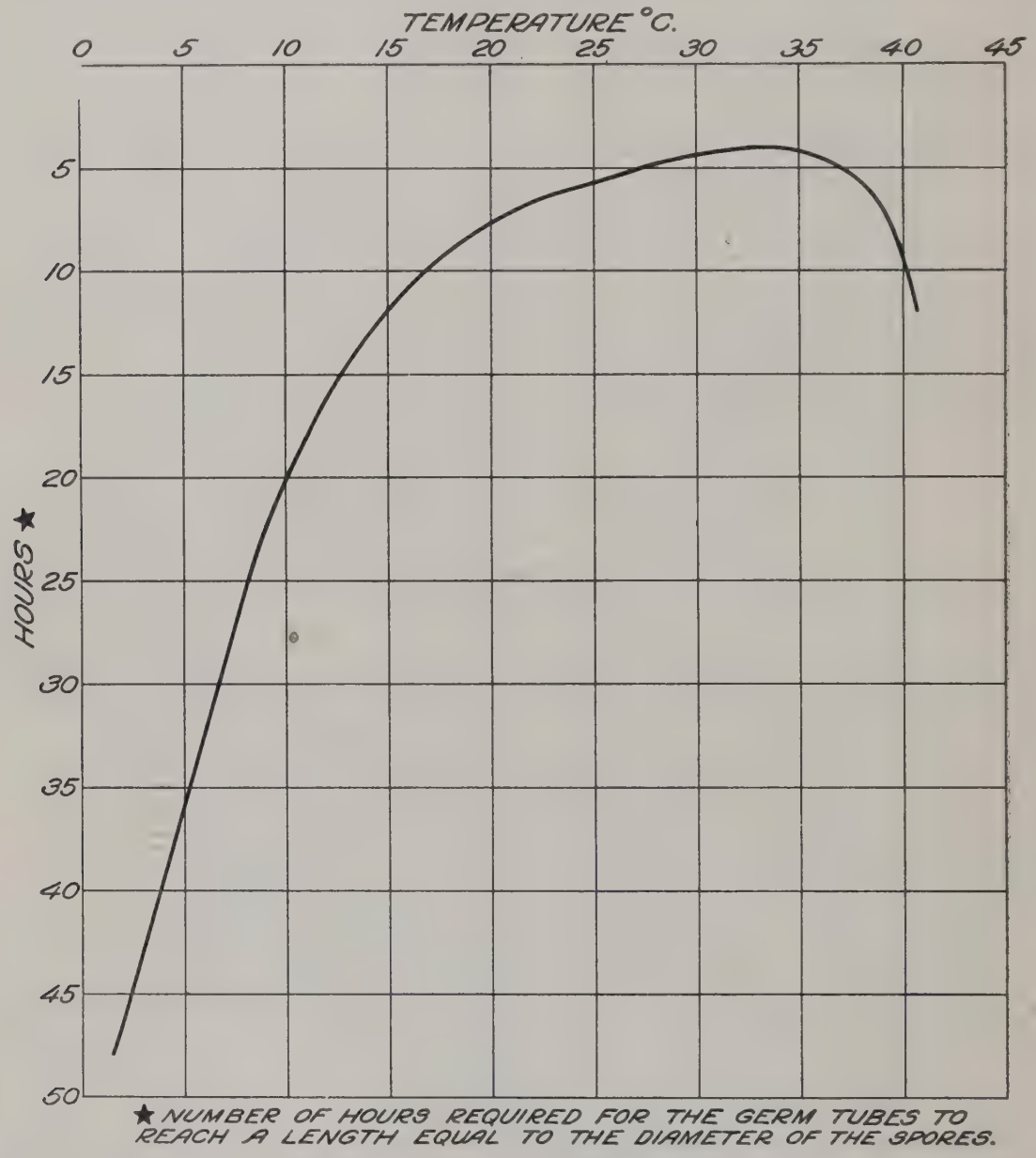


FIG. 1.—Curve showing the time in hours required for the spores to produce germ tubes equal in length to the diameter of the spores when incubated in sweet-potato decoction at different temperatures

No sharp optimum was noticeable for the first four days, but it became more pronounced thereafter. The optimum temperature tried was 26.8° C., although growth was only slightly less rapid at 22.5°, the next lower temperature. Above 26.8° the growth rate dropped abruptly. The optimum for mycelial growth lies somewhere between 25° and 27°, which is several degrees lower than that for spore germination. The optimum remained the

days at 38°, but a fair growth was made at 36°. Weimer and Harter (*18*) found that the more rapid-growing species of Rhizopus reached the highest thermal point of growth in the first 24 hours. Edson and Shapovalov (*6*) also found this to be the case with the fungi which they studied. However, as already pointed out, the brownrot fungus did not make a measurable amount of growth at the maximum temperature for several days. Never-

theless, this fungus grows very slowly at high temperatures and no doubt had made some growth several hours before a measurable disk was formed. This was also true as the minimum temperature was approached. No growth was evident at 2°, the lowest temperature tried, until after the eighth day. However, 5 and 13 mm. of growth were made at this temperature in 10 and 15 days, respectively. The minimum for growth is somewhere below 2°. Here again it is quite evident that the fungus can make considerable growth at the temperature existing in a refrigerator car in the week or two necessary for the shipment of the cauliflower from the Pacific coast to eastern markets.

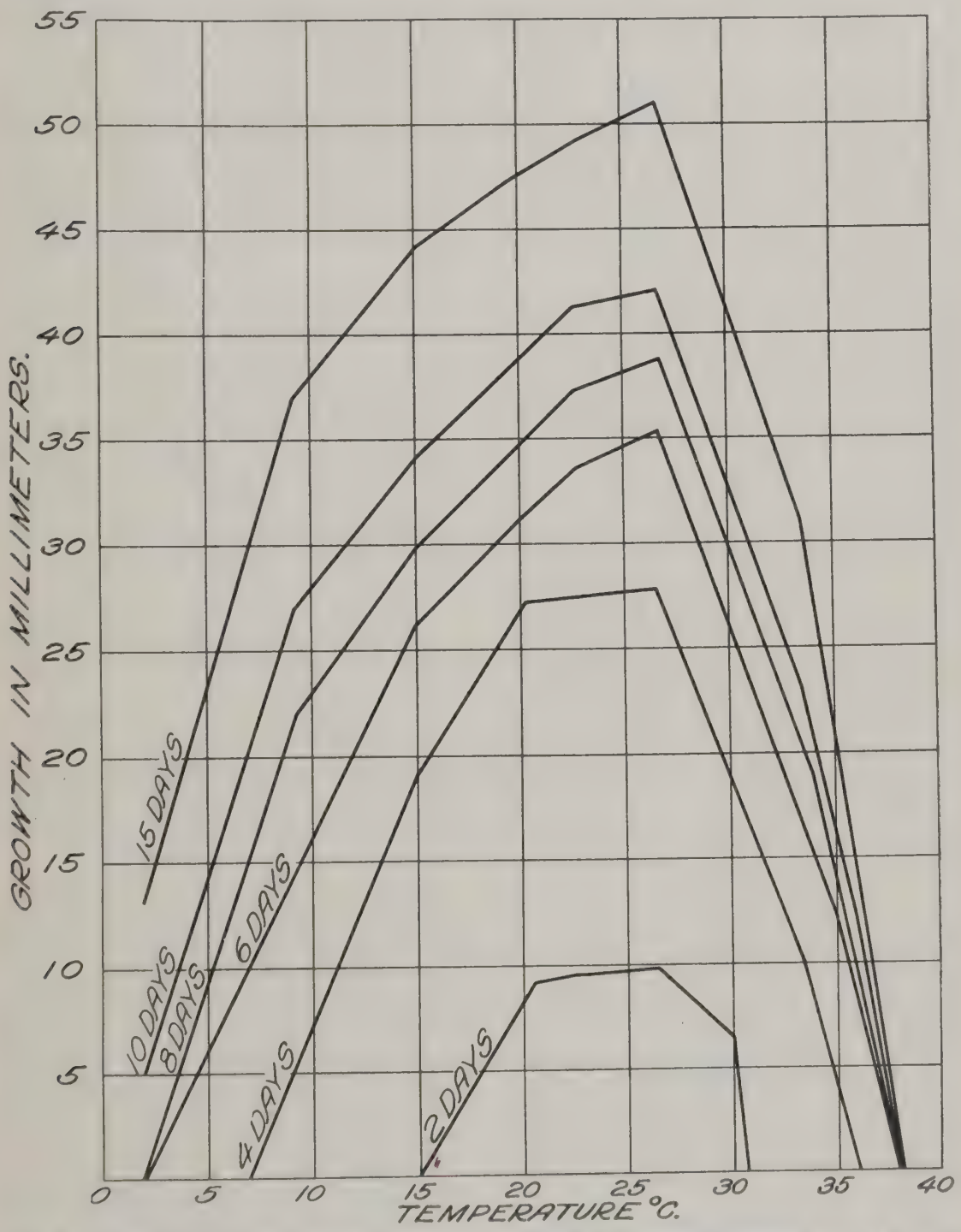


FIG. 2.—Graphs showing the diameter of the mycelial disks of *Alternaria brassicae* growing in Petri dishes on Irish potato agar at various temperatures after 2, 4, 6, 8, 10, and 15 days

The optimum temperature for fruiting as determined in these experiments was between 26° and 28°, while the maximum and minimum were practically identical with these temperatures for growth.

THE EFFECT OF TEMPERATURE AND HUMIDITY ON INFECTION

Young detached cauliflower leaves were employed to determine the influence of temperature upon infection. Because of continued catabolic activities and the retardation of anabolism, the leaves, after being removed from the plant, no doubt changed physiologically rather rapidly, especially at the higher temperatures. But, uninoculated control leaves at all but the highest temperatures remained turgid and retained their normal green color for several days longer than was necessary to obtain the desired results. Regardless of the change in the detached leaves, they more nearly approximated the normal plant than would any artificial culture medium which might have been used. The leaves were placed on moist cotton in small moist chambers in incubators at temperatures of 7°, 10°, 14°, 17°, 20°, 22°, 23.5°, 25.6°, 30°, 31°, 32.5°, 34°, 35.5°, 36.8°, and 38.2° C. The experiment was repeated with similar results. Abundant infection was evident after two days at all temperatures from 10° to 35.5° inclusive. Distinct lesions were present at 7° three days later, but no infection took place at 36.8° or above. The maximum for infection was about 36° since infection was obtained at 35.5° but none at 36.8°. The optimum for infection lay between 28° and 31°. Although there was not much difference in the number of lesions, they differed somewhat in size, being largest at the optimum and gradually becoming smaller above and below this temperature. At the optimum temperature aerial mycelium grew over the surface and the leaves gradually became yellow, while at the other temperatures the formation of aerial hyphae and the yellowing of the leaves was considerably slower. The optimum for infection of detached leaves under the conditions of these experiments was slightly higher than that for the growth of the mycelium on Irish potato agar and somewhat lower than that obtained for the germination of the spores.

Several experiments were conducted to determine the effect of temperature on infection and on the development of brownrot on the curd of cauliflower. The cauliflowers used were obtained from the city market at Washington, D. C., or were grown at the Arlington Experiment Farm at Rosslyn, Va. The curds were rinsed in tap water, placed in moist chambers on moist filter paper, sprayed with a heavy suspension of spores of *Alternaria brassicae* in distilled water, and then placed in a series of incubators whose temperatures ranged from 1.8° to 37.2° C. The incubators used were the same ones used in the spore germination and mycelial growth studies. Following are details of the effect of temperature on the infection of cauliflower curds by *Alternaria brassicae* and the development of brownrot:

At 1.8° infection became apparent in 5 to 7 days. After 14 days the lesions were chestnut brown, about 1 mm. in diameter and 0.5 mm. deep. At the end of 35 days the lesions had changed to an olivaceous color, due to the formation of spores. They were about 1 mm. in diameter by 1 to 2 mm. in depth.

At 7° numerous minute light brown lesions were apparent in 4 to 5 days. These were about 1 mm. in diameter by 1 to 2 mm. in depth in 19 days. In 35 days the lesions were 1 cm. in diameter by 1 to 1.5 cm. in depth.

At 9.5° abundant infection became evident in 3 days; these lesions were about 0.5 mm. in diameter and slightly more developed in 10 days. In 35 days the lesions were 2 to 3 cm. in depth and about half of the curd was involved.

At 10.5° infection became evident in 3 days and typical brownrot lesions 0.5 to 1.5 cm. in diameter developed in 20 days.

At 11° abundant but very small and superficial lesions became evident in 3 days. Tissues decayed to a depth of 0.5 to 1 mm. in 10 days. Practically the entire curd was involved in 35 days, the tissues being affected to a depth of 2 to 3 cm.

At 15.5° infection became evident in 3 days; the lesions were still quite small and superficial in 7 days, three-fourths of the curd being affected in 14 days.

At 18° lesions 0.5 mm. in diameter were evident in 48 hours, lesions 1 to 1.5 cm. in diameter were evident in 7 days, and the curd three-fourths involved in 14 days, the tissues being decayed to a depth of 1 cm. at the end of this time.

At 19.5° infection was just apparent in 24 hours; the lesions reaching 1 to 3 mm. in diameter in 3 days.

At 22.5° lesions were abundant but very small in 24 hours, about one-half of the curd being involved in 4 days. Nearly the entire curd was affected, the decay reaching a depth of 5 to 8 mm. in 7 days.

At 26.8° lesions were abundant in 24 hours and curd two-thirds involved in 4 days. Nearly the entire curd was affected, decay being 1 cm. deep in 7 days.

At 28.5° lesions were abundant in 24 hours. These were 1 mm. in diameter in 3 days, at which time bacterial rot was also present. The curd was nearly completely decayed by bacteria in 7 days.

At 30.7° lesions were abundant in 24 hours. These were about 1 mm. in diameter but were being obscured by bacteria in 2 days.

At 32° a few brownrot lesions, poorly developed, were evident in 3 days. Bacterial rot was present.

At 34.7° no brownrot developed in 3 days. The curd was decayed by bacteria.

At 37.2° no brownrot developed in 3 days. The curd was decayed by bacteria and Rhizopus.

Infection took place at all of the temperatures tried except 34.7° and 37.2° C. The disease developed more slowly at the lower than at the higher temperatures. The optimum temperature for infection lies somewhere between 25° and 30°. However, the optimum development of the disease occurred at about 25° since at the higher temperatures the heads were soon infected and decayed by bacteria. The number of lesions formed at any of the temperatures varied little except perhaps at 32°, where they were fewer. The infected areas in all cases were light brown in the early stages, gradually becoming darker, until they were olivaceous to nearly chestnut brown in color. The time necessary for the development of the aerial mycelium and spores which gave the olivaceous to brown color to the lesions varied with the temperature from about two days at the optimum to about 30 days at 1.8°. At 1.8° infection did not become apparent for from 5 to 7 days, at which time the lesions appeared as very minute lightbrown discolorations just visible to the unaided eye. Little progress had been made by the disease at the end of 19 days, as was indicated by a slight darkening in the color of the infected tissue. After 35 days the lesions had begun to take on the characteristic olivaceous color due to the presence of dark-colored aerial hyphae and spores. Nevertheless, at this time the decay had not penetrated over 1 to 2 mm. deep.

Infection was apparent in from 4 to 5 days at 7° and in 19 days the decay had extended into the tissue 1 to 2 mm. However, in 35 days the lesions were about 1 cm. in diameter and 1 to 1.5 cm. deep. At 9.5° to 15.5°, 18°, and 19.5° the maximum number of infections became evident in 3, 2, and 1 days, respectively. Although little actual decay was caused by this disease in two weeks below 10°, no doubt some decrease in the commercial value of the infected cauliflowers resulted from the browning of the curd.

The results of the experiments discussed above show quite definitely to what extent temperature may affect the development of brownrot on cauliflower under conditions of high humidity. An experiment was conducted in which the cauliflowers were sprayed with a heavy suspension of spores in water with an atomizer and then placed in wooden crates and held in large refrigerator rooms in a cold-storage house. The curds were fairly well enveloped by leaves. The temperatures of the rooms were 1°, 2.5°, 2.5°, and 4.5°, with a variation of perhaps 1° C. and a relative humidity of 81, 74, 91, and 87 per cent, respectively. Infection was visible in 10 days at all of the temperatures. But only a few lesions developed and these were still small at the end of a month. Under these conditions comparatively little damage resulted from this disease. This experiment was repeated later, the method being varied only in that the cauliflowers were placed in moist chambers with the expectation that the humidity of the air about the heads would thereby be raised. In this case the number of infections was greatly increased and the damage was proportionally greater. However, the fungus had not penetrated the tissues more than 0.5 cm. at any of the temperatures, there being little apparent difference in the stage of decay on the heads held at the different temperatures after five weeks.

EFFECT OF THE FUNGUS ON THE HOST

The blossoms of the cauliflower attacked by *A. brassicae* at first turn brown and take on a water-soaked appearance. This latter condition is especially noticeable in the larger branches of the curd. Very soon the lesions become olivaceous in color because of the presence of dark-colored aerial mycelium and spores. The mycelium is at first intercellular but later penetrates the cell walls and the diseased tissue is permeated with hyphae. The affected tissue is somewhat softened, although not so much so as in the bacterial rot. The decayed tissues lose water rather rapidly, so that the older lesions often have a dry and shriveled appearance. The fungus enters the leaves through the stomata and kills the tissues. This was demonstrated by placing drops of a suspension of spores upon a leaf in a moist

chamber. After 48 hours the inoculated spots were removed, fixed, dehydrated, infiltrated with paraffin, and sectioned. No evidence of direct penetration of the cell wall was seen. The cells showed a change in their reaction to stains very soon after the germ tubes had entered the stomata of the leaf. The cells soon died and the tissues affected turned dark. The extent to which the fungus spreads in the leaf varies greatly. It may form only a small dark spot about a millimeter in diameter, or it may spread throughout and kill a large area of the leaf.

PHYSIOLOGY

Chemical analyses (*5*) have shown that cauliflower contains the following carbohydrates: Cellulose, glucose, fructose, pentosans, and methyl pentosans. Dochnowski and Tollens (*5*) could not detect the presence of cane sugar but noted the existence of some hydrolyzable carbohydrates. On the other hand, Busolt (*2*) was unable to demonstrate the presence of glucose in cauliflower tissue but found mannit instead. Experiments were conducted to learn something of the carbohydrates which may serve as a source of carbon for *A. brassicae*. The carbohydrates tested were 5 and 10 per cent glucose, 1 per cent Irish potato starch, 5 per cent cane sugar, 5 per cent gum arabic and cellulose (dissolved and reprecipitated according to the method described by Scales (*15*)). Twelve 100 cc. Erlenmeyer flasks each containing 30 cc. of a modified Czapek's solution with the above carbohydrates substituted as a source of carbon were prepared and inoculated with a loop of a suspension of spores in sterile distilled water. The Czapek's solution was modified by the substitution of ammonium nitrate for sodium nitrate, as was found advantageous for the growth of Rhizopus by Weimer and Harter (*17*). One set of flasks had no carbohydrate present in order to determine if the fungus would grow on this solution without a carbohydrate being added. Ten of the 12 flasks of each set were inoculated and the two remaining held as controls. The flasks were held at about 30° in the dark for two weeks, after which observations were made on the amount and character of mycelial growth formed. There was not a sufficient amount of growth made in any of the flasks for dry-weight determinations. The presence of substances which did not go completely into solution, cellulose, starch, and gum arabic in particular made it impracticable to separate the mycelium from the medium by filtration; hence attempts to obtain the dry weight of the mycelium formed in each case for purposes of comparison were abandoned. Careful observations were made, however, which showed that practically no growth took place where carbon was not supplied. Glucose and cane sugar proved to be the best sources of carbon tried, followed closely by starch. Judging from the growth made, gum arabic can also serve as a source of carbon, but not nearly so effectively as the three substances mentioned above.

No more growth was made on the cellulose medium than in the controls where no carbon was present, which indicates that cellulose in this form can not be utilized by the fungus. In order to learn whether this fungus can hydrolize the starch and cane sugar, samples of the control solutions as well as those on which the fungus had grown were tested with Fehling's solution for the presence of reducing sugars. These tests were only comparative. Equal amounts of the culture media and of Fehling's solution (20 cc.) were used and the solutions were boiled approximately the same length of time in each case. The control cane-sugar solution showed only a trace of reduction, while some of the same solution on which the fungus had grown gave a heavy precipitate with Fehling's solution, there being more than enough reducing sugar present to reduce all of the copper. This indicates that the fungus produces a very active invertase. Similar tests with the solutions containing starch showed that there was no reducing sugar present in the control and only a very little in the inoculated solutions. Seemingly, the amylase produced, if any, was not sufficient to hydrolyze the starch much in excess of the needs of the fungus.

Since this fungus produces a rot of the cauliflower curd it is of interest to know whether an enzym capable of dissolving the middle lamellae is produced under artificial culture conditions. In order to determine this point, about 25 cc. of each of the above-mentioned solutions were placed in small flasks and small pieces of cauliflower curd and leaf and sweet potato were added. The sweet potato added was in the form of circular disks about 1 cm. in diameter by 0.5 mm. thick, cut from the fleshy root. The flasks were held at 45°. Five cc. of toluol were added to each flask as an antiseptic. Observations were made from time to time but no signs of maceration were evident in any case in 72 hours.

The above experiments indicate that a strong invertase; a weak amylase, if any; no cytase; and no pectinase were produced. To obtain further confirmation regarding the production of these enzyms by *A. brassicae*, fifty 200-cc. flasks, each containing 50 cc. of sweet-potato decoction, and a like number containing cabbage decoction, were prepared and inoculated. Several uninoculated flasks were held as controls. The Czapek's solution used in the earlier experiments did not appear to contain all the substances necessary for the best development of the fungus, hence these vegetable decoctions were used and proved to be very satisfactory; 500 gm. of the vegetables per liter were used in preparing the decoctions. Part of the inoculated flasks were held at 31° in the dark and the remainder were placed at room temperature (25°) and in diffused light. After 12 days a thick mycelial felt was present on all of the inoculated flasks. The amount of growth on these media was very much better than that made on Czapek's solution. The mycelial felt was very dark olive to almost black in color. Spores were present in great abundance. It might be added here that the fungus seems to sporulate even under the most adverse conditions, spores having been formed in considerable quantities even in Czapek's solution without carbon. Furthermore, it was found in the temperature experiments previously recorded that spores were formed at temperatures so high that they were extremely injurious to vegetative growth. In fact, sporulation seems to take place under any condition which will permit growth.

The felts were removed from the sweet potato and cabbage decoctions, both those in the light and in the dark. Each set was kept separate throughout. These felts were washed in running tap water for a few minutes to remove all traces of sugar and were then treated with acetone and ether in the manner described by Harter and Weimer (*10*). The hyphae were then weighed out in 0.5 gm. samples, each being placed in a small Erlenmeyer flask. Twenty-five cc. of distilled water were added to two flasks of each set. To these flasks were added disks, 1 cm. in diameter by 0.5 mm. thick, of sweet potato, carrot, and Irish potato and similar disks cut from the laminae of cauliflower leaves as well as small portions of the cauliflower curd. Twenty-five cc. of 0.75 per cent cane-sugar solution were added to each of two flasks containing hyphae grown on sweet-potato decoction in the light, to each of two flasks containing hyphae grown on sweet potato decoction in the dark, and to each of two flasks containing hyphae grown on cabbage decoction in the dark. Twenty-five cc. of a 0.75 per cent starch paste and 25 cc. of a cellulose suspension were added to each of two flasks containing hyphae from the same sources. Likewise, flasks containing the same amount of hyphae from the same sources with 25 cc. of distilled water added were held as controls. Samples of the cane-sugar solution, starch paste, and cellulose suspension were also held as controls; 5 cc. of toluol were added to all of the flasks as an antiseptic, and they were then held at 45°.

No maceration was evident in any of the flasks containing the hyphae and portion of vegetable in 96 hours, at which time the experiment was discontinued. Here, as in the former test, the presence of pectinase could not be demonstrated.

After 24 hours the flasks to which the cane sugar had been added were steamed and their contents tested for reducing sugars by the Clark method (*4*). At the end of 30 hours the flasks to which the starch paste and cellulose had been added were similarly treated and tested. The control flasks containing samples of the cane sugar, starch paste, and cellulose were also tested. Likewise, the control flasks containing hyphae and water were tested in the same way. The purpose of holding the hyphae in water was to determine the reducing sugar formed as a result of autolysis of the fungus itself. The average amount of reducing sugars present in the two flasks of each set minus that formed by autolysis and that in the original solutions are given in Table III. The data presented there show that a considerable quantity of both the cane sugar and the starch was hydrolyzed by the enzyms contained in the hyphae, denoting that invertase and amylase were produced by this fungus when growing on either sweet-potato or cabbage decoction. The number of tests made was not large enough to make the figures very accurate from a quantitative standpoint, but they are of value in that they show that considerable reducing sugar was produced in both the cane-sugar and starch solutions. Apparently, slightly more invertase was produced by the fungus when growing on cabbage than on sweet-potato decoction. However, this difference in the results obtained may be within the range of experimental error. On the other hand the reverse seemed to be true of amylase. Light appeared to have a detrimental effect on both enzyms.

It has been shown by Weimer and Harter (*19*) that acids were formed in the solutions on which species of Rhizopus grew in sufficient quantities to cause maceration of the sweet-potato disks. In order to be sure that the acidity or alkalinity of the media on which *A. brassicae* had grown was not such as to interfere with the enzym studies, the hydrogen-ion concentrations of the solutions were determined (electrometrically) and are given in Table IV. The figures in this table show that the cabbage decoction underwent slightly greater change in hydrogen-ion concentration than did the sweet-potato decoction. The change seemed to be slightly greater in the solutions on which the fungus had grown in the light. The data show that the hydrogen-ion concentration of none of the solutions was such as seriously to interfere with the pectinase tests made.

LIFE HISTORY

There seems to be little doubt that the causal organism (*A. brassicae*) lives over winter in the soil and in diseased parts of the host left on the ground. The results of experiments have shown that the spores will not only live but will germinate and grow at 2°. Although no experiments have been conducted to determine the minimum temperature which the spores will survive, there is every reason to believe that they will stand quite low temperatures. This is supported by the fact that this disease is prevalent in sections of the country where very low temperatures are experienced each winter. There is also the possibility that the fungus lives over winter on the seed as is shown by the following experiment:

Cauliflower seeds were dipped into a suspension of spores from a virulent culture of *A. brassicae*, then dried and stored in a drawer of a laboratory desk for eight months. Tests showed that some of the spores were then still capable of germinating. Some of the seeds dipped into the spore suspension were planted in a pan of sterilized soil in the greenhouse. A pan filled with some of the same soil was sown with seed from the same lot which had not been inoculated, as a control. A damping-off of the seedlings occurred in the pan sown with inoculated seed and *A. brassicae* was recovered from the diseased tissue a couple of centimeters above the surface of the ground. No damping-off occurred in the control pan. This experiment indicates that the disease may develop in the seed bed from the spores on the seed. In addition to attacking leaves, stems, and heads of cauliflower, the fungus also causes a spotting of the seed pods. It has been isolated from seeds taken from beneath the lesions on partly mature pods. Spores of the fungus have been seen on seeds taken from pods which had not yet begun to dehisce. This shows that the fungus can grow through the pods and infect the seeds. The fungus may be carried to the seed beds with such seeds and a damping-off of the seedlings produced. Spores are formed abundantly on the dead seedlings and serve as a source of inoculum for other plants. A few plants having small lesions on the lower leaves have been seen at transplanting time, showing that the disease may be carried from the seed bed to the field on the seedlings. This has not been seen very frequently and probably occurs only under conditions extremely favorable for the development of the fungus.

Inoculation experiments conducted in the greenhouse showed that the incubation period in the leaves was from 24 to 48 hours. The time necessary for infection to become evident varies with the temperature and humidity. Cauliflower plants with heads from 5 to 10 cm. in diameter growing in the field were inoculated on June 18, 1921. Infection took place and well developed lesions were present both on the leaves and curds in six days. At this time typical Alternaria spores were present in abundance both on the leafspots and the brownrot lesions. These spores served as a source of inoculum so that by the end of the season the disease was present on many of the neighboring uninoculated plants. The spores are easily separated from their sporophores, so that ample opportunity is thus afforded for the disease to become widespread in a comparatively short time, provided conditions are favorable for dissemination and infection.

MORPHOLOGY

The mycelium permeates the tissues of the head and leaf in all directions from the point of infection passing between and through the cells. The hyphae are hyaline at first, later becoming brownish to olivaceous. In culture mycelial development is suppressed, but on or in the curd of the cauliflower long profusely branched threads with numerous septations are developed. The hyphae vary in diameter on different substrates, ranging from 1.5 to 7.5 μ. They soon come to the surface of the host, finding an exit through stomata or wounds.

Under humid conditions the surface is soon covered with conidiophores bearing an abundance of spores. The conidiophores are short, olivaceous in color, usually concolorous with the spore, septate and branched. They are 5 to 7.5 μ wide by about 35 to 45 μ long. The spores are nearly linear to obclavate in shape and are borne on the conidiophores in chains, as many as 8 to 10 in a chain being quite common. The spores are produced abundantly both in culture and on the host. They are olivaceous to brown in color and together with the conidiophores impart the characteristic olivaceous color to the lesions on the host. They are smooth at first, becoming roughened with age, and have as many as 10 horizontal septations but few vertical ones. Spores from old cultures often have more septations and are thicker in proportion to their length and more obclavate than those in fresh cultures or those from fresh lesions. Spores with more than six horizontal septations are not numerous. Various spore types are shown in Figure 3.

FIG. 3.—Various types of spores of *Alternaria brassicae*. $\times$ 1,000.

The spores taper somewhat toward the apex. The apical cell often is more or less rectangular in cross section, the outermost wall, however, being shorter than the wall contiguous with the penultimate cell. The basal cell is somewhat rounded. The maximum and minimum measurements for spores with different numbers of septations taken both from culture and from the host are as follows: 1, septate 6.5–7.5$\times$11–13 μ; 2, septate 7.5–9.5$\times$18.5–20.6 μ; 3, septate 9–11.2$\times$26–41.25 μ; 4, septate 9–11.25$\times$39.5–45.5 μ; 5, septate 9–13.9$\times$37.5–45 μ; 6, septate 9–11.25$\times$35.6–45 μ; 7, septate 13–16.8$\times$56.25–65 μ; 8, septate 11.25–16.8$\times$50–75 μ. The perfect stage of this fungus is unknown.

Elliott (*7*) discusses what he terms secondary development in Alternaria spores. This was also noticed by the writer. In the young cultures the spores are regular and smooth with few vertical cross walls. As the spores grow older the constrictions at the septa become deeper, the cells round off, the spores become darker in color, and the walls become roughened. This variation of the spore size and character has led to considerable confusion in the determination of *A. brassicae*.

CONTROL OF THE DISEASE

Alternaria leafspot of cauliflower and cabbage is of comparatively little importance during most seasons, so that ordinarily no remedial measures are necessary. However, spraying with Bordeaux mixture 4–4–50 with a sticker added is recommended in case of a severe outbreak (*16*).

The results of other workers seem to indicate that this disease may be held in check somewhat by treating the seed. Chupp (*3*) reports a 100 per cent killing of Alternaria on the seeds of cabbage by treating them with hot water at 56° for 10 minutes. However, the fungus could still be isolated from seeds treated with mercuric chloride. He likewise states that Walker found that treating cabbage seed with hot water at 50° for 30 minutes or 55° for 10 minutes will completely eliminate Alternaria.

As stated above, the disease on cauliflower plants growing in the field is not

sufficiently serious during most seasons to justify the application of control measures. However, a large number of spores may be produced on a comparatively few spots. These spores may infect the curd or may lie dormant until the heads are in transit. An attempt should be made to discard all heads with apparent lesions when the cauliflower is being crated, since such heads will have little if any market value after several days in transit. However, many inconspicuous lesions will be overlooked and also many more may originate during the shipping period. That the disease may be expected to develop during transit is shown by the fact that in the writer's experiments infection was evident at about 2° C. in from 5 to 7 days. The lesions, however, developed rather slowly, being about 1 mm. in diameter by 0.5 mm. in depth in 14 days under conditions of high humidity. At 7° the lesions were evident in from 4 to 5 days and had developed to 1 mm. in diameter by 1 to 2 mm. in depth in 19 days. These data indicated that it is possible for the disease to make considerable headway in the 10 days or thereabouts which must necessarily intervene between the time California cauliflower is placed in the car and the time it reaches its destination. The amount of loss caused depends upon the amount of infection and the number of spores on the heads at the time they are loaded into the car, as well as the temperature and humidity maintained in the car throughout the transportation period. In another experiment cauliflower inoculated and held at about 2° became infected, but the lesions were hardly visible to the unaided eye even after 20 days and would not have been recognized as such by the layman. In this experiment at temperatures varying from 6° to 7° C. the lesions were hardly recognizable at the end of 10 days and were still quite inconspicuous after 20 days.

From this and other experiments it seems that, although it is impracticable to hold cauliflower at a temperature which will inhibit infection during transportation, much can be accomplished by maintaining the temperature and relative humidity as low as possible. If the temperature is held at 5° C. or below throughout the transportation period of, say, 6 to 12 days, little injury will result from this disease. This is entirely practicable, as shown by tests with cantaloupes made by the Bureau of Agricultural Economics, United States Department of Agriculture (*12*). These showed that refrigerator cars loaded with cantaloupes at Brawley, Calif., did not reach a constant temperature level until about the end of the third day, after which a fairly uniform temperature was maintained. The temperatures in a couple of cars varied from about 1° to 6° C., depending on the part of the car in which the records were obtained. The cars were on the road 5 or 6 days and 12 days when en route to Chicago and to New York, respectively. Temperature records of other cars similarly loaded showed that after the cantaloupes became thoroughly cooled (about the third day) a temperature varying from about 4° to 10° C. could be maintained.

The cauliflower heads should be kept as cool and dry as possible after reaching their destination, and should reach the consumer without unnecessary delay. Precooling the cars and the cauliflower especially during warm weather before loading should be of material benefit in getting the temperature down and retarding the development of the disease during the early period of transportation.

The inspectors of the Bureau of Agricultural Economics reported the arrival of a car of cauliflower in Pittsburgh on November 21, 1921, which had been shipped from Orchard Park, N. J. About one-third of the curds showed brownrot in varying degrees of severity; some had only a few small spots, while others were almost entirely covered with them. The temperature of this car taken at the time the inspection was made was 8° at the bottom and 9° C. at the top. Although these figures are of little value in showing the temperature condition of the car during transit, the condition of the cauliflower indicates that the disease can develop in transit when hauled only a comparatively short distance if the conditions are favorable. No record of the time elapsing between the dates of loading and of unloading the car was obtained.

The data presented in this paper seem to justify the following recommendations for controlling the brownrot of cauliflower:

Prevent field inoculation and infection by keeping the disease under control on the growing plants. With this in view, practice seed treatment, sanitary seed-bed preparation, and crop rotation. If the disease becomes at all prevalent the plants should be sprayed with 4–4–50 Bordeaux mixture.

Pack and ship only cauliflower entirely free from the disease.

Ship in good refrigerator cars, which are kept at as low a temperature as possible (7° C. or below) throughout the transportation period by refilling

TABLE I.—*Amount of brownrot found by inspectors of the Bureau of Agricultural Economics on cauliflower in various shipments*

Date	Source	Market	Percentage of heads affected
Dec. 12, 1920	Los Angeles, Calif.	Chicago, Ill.	3–5
Mar. 23, 1920	do	Fort Worth, Tex.	50–100
Feb. 13, 1920	do	Chicago, Ill.	20–30
Feb. 7, 1920	do	do	100
Jan. 1, 1920	Compton, Calif.	do	0–20
Feb. 6, 1920	Bradentown, Fla.	do	80
Mar. 22, 1919	San Francisco, Calif.	New York, N. Y.	10

TABLE II.—*Detailed data on experiments conducted to prove the pathogenicity of A. brassicae isolated from different types of lesions on different hosts and from different sources*

Number of organism	Source of organism	Date of inoculation	Host	Part of plant used	Number of plants inoculated	Number of plants infected	Number of controls	Number of controls infected
		1921						
4746c	Cauliflower curd, Washington, D. C., city market.	Jan. 8	Cauliflower	Curd	2	2	1	0
4829	Pure line of 4746c	Mar. 12	do	do	2	2	1	0
4829	do	do	Cabbage	Head	2	2	1	0
4829	do	Mar. 14	Cauliflower	Seedlings	(a)	(b)	(c)	0
4829	do	Mar. 31	do	do	(d)	(b)	(a)	0
4829	do	do	do	Half-grown plants.	3	3	1	0
4829	do	do	Cabbage	do	3	3	2	0
4829	do	Apr. 4	Cauliflower	Curd	1	1	1	0
4828	Cauliflower leaf, California	do	do	do	1	1	1	0
4866a	Cauliflower curd, California	do	do	do	1	0	1	0
4829	Pure line of 4746c	June 2	do	Plants in field, curd.	7	7	7	0
4828	Cauliflower leaf, California	June 18	do	do	5	5	5	0
4829	Pure line of 4746c	do	do	do	10	10	5	0
4829	do	do	do	do	8	8	6	0
4828	Cauliflower leaf, California	do	Cabbage	Plants in field	5	5	6	0
4829	Pure line of 4746c	July 4	do	do	10	10	5	0
4828	Cauliflower leaf, California	Nov. 10	Cauliflower	Curd	1	1	1	0
4829	Pure line of 4746c	do	do	do	1	1	1	0
4973d	Cauliflower curd, Washington, D. C.	do	do	do	1	0	1	0
4866a	Cauliflower curd, California	do	do	do	1	1	1	0
5047	Washington, D. C., city market.	do	do	do	1	1	1	0
4997c	Cabbage leaf, Arlington, Va.	do	do	do	2	2	2	0
		1922						
5104	Cabbage from New York, in storage at Washington, D. C.	Feb. 22	do	Plant 1 foot high.	2	2	2	0
4829	Pure line of 4746c	do	do	do	2	2	2	0
4997c	Cabbage leaf, Arlington, Va.	do	do	do	2	2	(e)	0
4828	Cauliflower leaf, California	do	do	do	2	2	(e)	0
4973d	Cauliflower curd, Washington, D. C.	do	do	do	2	2	(e)	0
5093	Cabbage leaf	do	do	do	2	2	(e)	0
5091	Cauliflower curd, Florida	do	do	do	2	0	(e)	0
5095	Cauliflower leaf; not typical Alternaria spot.	do	do	do	2	0	(e)	0
5089	Cabbage leaf	do	do	do	2	0	(e)	0
4866a	Cauliflower curd, California	do	do	do				

[a] 2 pans. [b] Large number. [c] 1 pan. [d] 4 pans. [e] Same control as above.

TABLE III.—*The quantity of reducing sugars in mg. per 10 cc. resulting from the hydrolysis of cane sugar, starch, and cellulose by 0.5 gm. of hyphae of A. brassicae in 25 cc. of solution*

Substances tested	Hyphae grown on sweet-potato decoction		Hyphae grown on cabbage decoction in the dark
	In the dark	In the light	
0.75 per cent cane-sugar solution	91.38	82.66	95.58
0.75 per cent starch paste	68.80	58.14	61.05
Cellulose	00.00	00.00	00.00

TABLE IV.—*The hydrogen-ion concentration of cabbage and sweet-potato decoctions on which A. brassicae had grown for 12 days and of the uninoculated controls*

Solutions	Treatment	P_H
Cabbage decoction	Uninoculated control, held in the dark	5.24
Do	Uninoculated control, held in the light	5.01
Do	Inoculated, held in the dark	7.27
Do	Inoculated, held in the light	7.54
Sweet-potato decoction	Uninoculated control, held in the dark	4.96
Do	Uninoculated control, held in the light	4.96
Do	Inoculated, held in the dark	6.46
Do	Inoculated, held in the light	6.58

the ice bunkers at frequent intervals. The addition of salt to the ice in the bunkers and the placing of ice in and over the load will help to keep the temperature down. Precooling the cars as well as the cauliflower before loading should prove beneficial, especially when shipments are made during warm weather.

SUMMARY

(1) The leafspot of cauliflower caused by *Alternaria brassicae* has been studied, the pathogenicity of the casual fungus has been established, and the symptoms produced on plants of different ages and under different conditions described.

(2) A decay of the curd of cauliflower, called "brownrot", is also produced by *A. brassicae*. This disease of the head is found only when the cauliflower is overmature or when the head has been cut from the stalk. The disease is most destructive on cauliflower heads in transit where they are decayed or discolored, so that their market value is greatly reduced.

(3) *A. brassicae* has an optimum temperature for spore germination of 33° to 35° C. The maximum temperature for spore germination lies somewhere between 40° and 46° and the minimum is below 1.5°.

(4) The optimum temperature for the growth of the mycelium in Petri dishes on Irish potato agar lies between 25° and 27°. The fungus made some growth at 36° but none at 38°. No growth was evident at 2°, the lowest temperature tried, until after the eighth day. The minimum for growth is somewhere below 2°, although growth is very slow at this temperature.

(5) The maximum temperature for infection of detached cauliflower leaves, artificially inoculated and held in moist chambers at various temperatures, was about 36°. The optimum for infection under the same conditions lay between 28° and 31°. Infection was evident at 7° in five days.

(6) The optimum temperature for infection of the heads inoculated and held at various constant temperatures in moist chambers was between 25° and 30°. No infection took place at 34.7° or above. Infection did not become apparent at 1.8° for from 5 to 7 days, at which time the lesions appeared as very minute light-brown discolorations just visible to the unaided eye. The disease had developed but little at the end of 19 days. Infection was apparent in from 4 to 5 days at 7°, and in 19 days the decay extended into the tissue 1 to 2 mm. Although little actual decay was caused by this disease in two weeks below 10°, some decrease in the commercial value of the infected cauliflower resulted from the browning of the curds. The amount of infection and the rate of development of the disease increase as the humidity of the surrounding air is raised.

(7) Enzym studies showed that *A. brassicae* produced invertase and amylase but not pectinase or cytase, at least in demonstrable amounts, when growing on sweet-potato and on cabbage decoctions. These decoctions were made less acid by the fungus.

(8) *A. brassicae* attacks the leaves, stems of seedlings, heads, seed pods, and seeds of cauliflower. It has been isolated from seeds taken from within infected immature pods, showing that it can grow through the pod into the seeds. The fungus can be carried to the seed bed on the seeds, where it can cause damping-off of the young seedlings. Spores are produced abundantly on seedlings killed in this manner and these may serve as a source of further infection. The fungus can no doubt also live from one season to another in the soil.

(9) Suggestions are made for controlling the disease.

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THE DUSTFALL OF FEBRUARY 13, 1923 [1]

By Alexander N. Winchell, *Professor of Mineralogy and Petrology, University of Wisconsin*, and Eric R. Miller, *Meteorologist, United States Weather Bureau*

INTRODUCTION

Dustfalls seem relatively rare. During the past six years the catch of every rain and snow at Madison, Wis., has been filtered, but samples from only four dustfalls have been obtained. Except in those four well-defined cases, the solid precipitates were very small in amount and consisted mostly of soot and other products of combustion. Reports on the dustfall of March 9, 1918, and of a second on March 19, 1920, have already been published by the writers (*11, 12*).[2] The third dustfall, on February 13, 1923, is discussed in the present paper. The unusually complete samples afforded by the fourth dustfall, on March 29, 1924, are now being prepared for analysis.

DESCRIPTION OF THE DUSTFALL

The dustfall of February 13, 1923, differed from the other three in that the region of deflation extended nearer to Madison, Wis., on this occasion. The dust fell at Madison in a snowfall of 0.6 inch which fell as the center of Low No. IV passed over. Figure 1 shows the track of this storm. At Madison the accompanying precipitation lasted from 1.30 to 4.30 p. m. The direction and velocity of the wind during this time were as follows:

Time (p. m.)	*Direction*	*Miles per hour*
1 to 2	Northeast	5
2 to 3	West	7
3 to 4	Northwest	10
4 to 5	West	27

Velocities averaging 25 miles per hour continued for the following 24 hours.

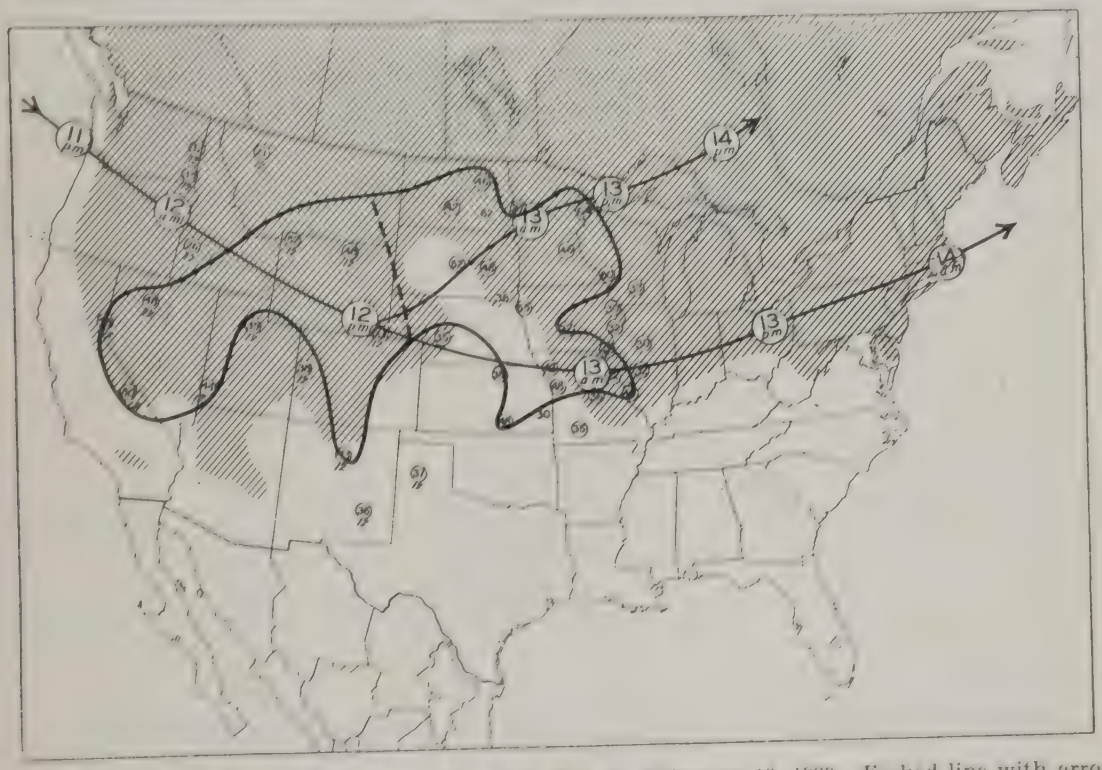

FIG. 1.—Shaded area shows extent of snow cover at 7 p. m. February 12, 1923. Forked line with arrowheads shows paths of centers of dust-bringing whirlwind. Numbers within small circles show maximum wind velocities in miles per hour. The day of occurrence was February 13 except at places marked 12 below the circle, where it occurred on the 12th. The closed line includes all places where the maximum velocity exceeded 40 miles per hour

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[2] Reference is made by number (italic) to "Literature cited," p. 450.

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The rapid rise of velocity after the passing of the storm center was produced by the steep barometric gradient between Low IV and High VIII which followed closely. These strong following winds characterized this storm all the way across the western half of the continent. The closed loop in Figure 1 shows the area within which the wind attained velocities exceeding 40 miles per hour at all stations. These velocities occurred west of the dividing line on February 12, and east of that line on February 13. The winds apparently blew the dust up into the air, where it was carried forward into and around the storm center, and there precipated with rain and snow. Air trajectories of this type have been traced by Shaw and Lempfert (*9, figs. 84 K and 86 B*).

Snow covered most of the region within the area of high winds, except the Missouri valley (fig 1). Trees and other tall vegetation exposed to the wind throughout the area doubtless contributed to the organic matter shown by the analyses. The dust-bearing snow was so badly drifted that no attempt was made to ascertain the weight of the dust per unit area. The samples used in the analyses were collected in the country west of Madison by W. S. Fusch and Dr. J. G. Dickson. Director Edwin B. Frost, of the Yerkes Observatory, also forwarded large samples obtained by sweeping the terrazzo floor of the observatory, into which the wind had blown dust through crevices.

PHYSICAL COMPOSITION

Prof. H. W. Stewart, of the Soils Department of the University of Wisconsin, made a mechanical analysis of the dust. His results are given in Table I.

The breaking of a tube prevented completion of the duplicate on the clay and silt separates. The "fine gravel," "coarse sand," and "medium sand" are practically all plant tissues and not mineral matter. There is some organic

TABLE I.—*Size of constituents of dustfalls in a 10-gm. sample of air-dried material*

Separates	Size	Per cent in duplicate parts		Average
		A	B	
Fine gravel	2.0–1.0	0.15	0.10	0.12
Coarse sand	1.0–0.5	0.40	0.41	0.41
Medium sand	0.5–0.25	1.10	0.98	1.04
Fine sand	0.25–0.1	4.37	3.74	4.06
Very fine sand	0.1–0.05	33.17	33.36	33.26
Coarse silt	0.05–0.025	14.15	----------	14.15
Medium silt	0.025–0.01	33.76	----------	33.76
Fine silt	0.01–0.0005	3.79	----------	3.79
Clay	Less than 0.005	10.60	----------	10.60
Total		101.49	----------	101.19

TABLE II.—*Size of constituents of dustfalls compared with that of loess*

Size	Percentages				
	A	B	C	D	E
<0.005	10.60	25.46	11.15	24.5	28.9
0.005–0.010	3.79	12.04	22.01		
0.010–0.025	33.76	44.60	56.17	66.5	63.9
0.025–0.050	14.15	11.47	5.99		
0.05–0.10	33.26	4.96	1.22	5.8	4.6
0.10–0.25	4.06	.82	1.04	1.0	1.3
0.25–0.50	1.04	.04	0.58	0.4	0.4
0.50–1.0	0.41	.02	0.29	1.2	0.6
1.0–2.0	0.12	.00	1.08	0.6	0.2
Total	101.19	99.41	99.53	100.0	99.9

A.—Dust from snowfall at Madison, Wis., Feb. 13, 1923.
B.—Average of three samples of dustfall at Madison, Wis., Mar. 19, 1920 (*12, p. 349*).
C.—Dustfall at Madison, Wis., Mar. 9, 1918 (*11, p. 602*).
D.—Loess, 3 feet below surface, Muscatine Co., Iowa (*3, p. 1848*).
E.—Loess, 3 feet below surface, Ringgold Co., Iowa (*2, p. 1918*).

matter in the other parts, but the percentage is very small.

It may be useful to compare these results with similar analyses of other dustfalls and of loess from the upper Mississippi valley, as shown in Table II and in Figure 2. Many other mechanical analyses of loess from the no doubt that part of it is actually present in the ferrous state. The only analysis of another American dustfall with which this can be compared is that of an earlier fall at Madison. As only a few satisfactory analyses of foreign dustfalls are available, all the good ones are included in Table IV.

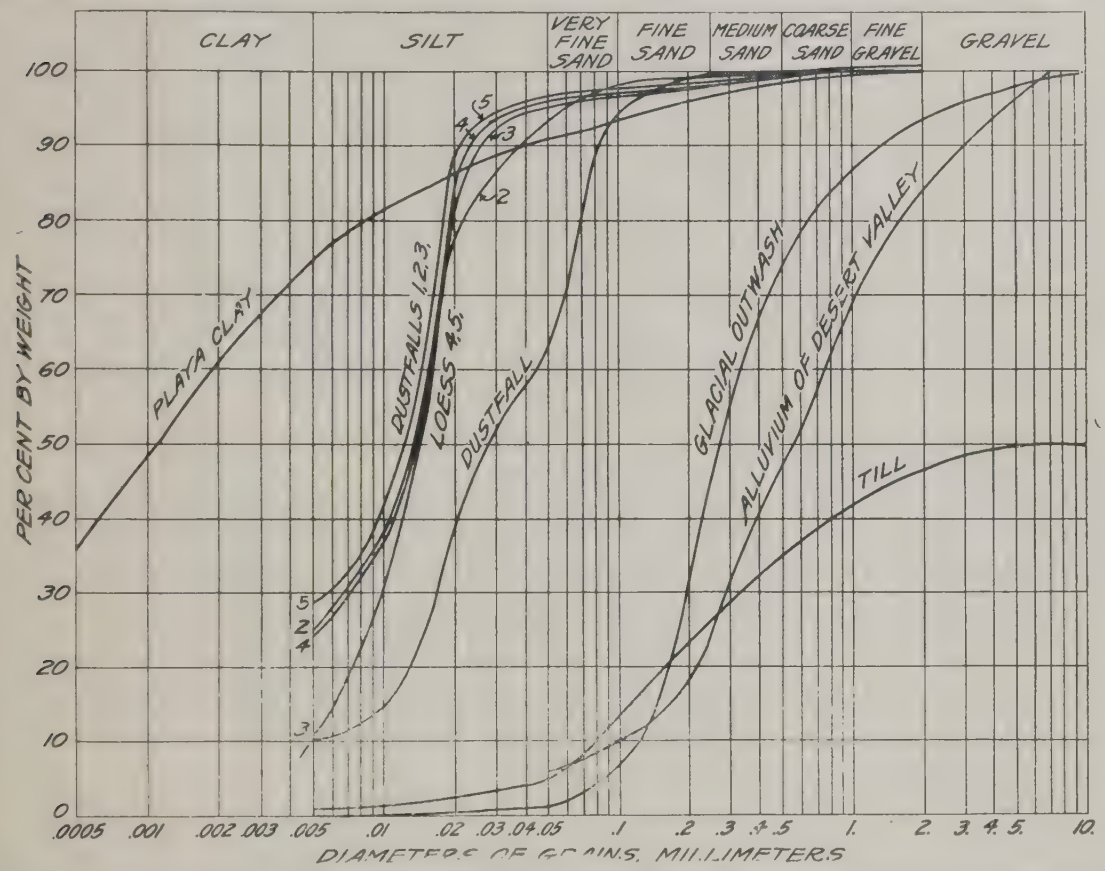

FIG. 2.—Mechanical composition of dustfalls and of natural soils. The ordinate shows the per cent by weight of all sizes smaller than the size indicated by the abscissa. Curves 1 to 5 refer to Table II. Other curves from Meinzer, Water Supply Paper 489

Mississippi Valley could be given, but they simply show that it varies considerably in the abundance of the various sizes. Increase of examples would not modify the main conclusion, which is that there is no essential difference in this respect between the material of dustfalls and that composing loess.

CHEMICAL COMPOSITION

Dr. E. J. Graul, of the Soils Department of the University of Wisconsin, made a determination of the alkalies in the dust. The main analyses were carried out in duplicate by Martin Tosterud, of the Chemistry Department of the University.[3] The results are shown in Table III. It was impossible to determine the state of oxidation of the iron in the dust because of organic matter. The small amount present was determined after complete oxidation, and is so reported, but there is

TABLE III.—*Chemical composition of the Madison dustfall of February 13, 1923 (dried at 105° C.)*

Constituents	Percentages		
SiO_2	71.75	71.70	71.73
Al_2O_3	7.84	7.85	7.85
Fe_2C_3	3.85	4.01	3.93
FeO			
MgO	1.01	.99	1.00
CaO	1.83	1.82	1.83
Na_2O	1.12	1.22	1.17
K_2O	2.15	2.06	2.11
H_2O (above 105° C.)	5.00	4.89	4.95
TiO_2	.48	.49	.49
P_2O_5	.17	.17	.17
MnO	.24	.17	.21
N	.37	.37	.37
Ignition [a]	3.57	3.65	3.61
	99.38	99.39	99.42
H_2O (below 105° C.)	1.65	1.66	1.66
H_2O (below 110° C.)	2.46	2.45	2.46

[a] Includes organic matter and CO_2, but not H_2O nor N.

[3] In order to further this work a grant of funds for chemical analyses was obtained from the research fund of the University of Wisconsin.

TABLE IV.—*Chemical composition of Madison dustfalls compared with that of foreign dustfalls*

Constituents	Percentages						
	A [a]	B	C	D	E	F	G
SiO_2	71.73	67.20	53.68	45.94	45.40	41.43	36.32
Al_2O_3	7.85	13.71	18.44	18.35	19.97	10.38	16.35
Fe_2O_3	3.93	2.17	6.54	6.57	7.03	9.19	6.08
FeO							
MgO	1.00	1.76	1.52	1.86	3.13	.92	2.21
CaO	1.83	1.74	.95	8.64	6.50	14.10	6.24
Na_2O	1.17	2.11	1.67	1.16	2.61	1.66	2.59
K_2O	2.11	2.30	2.58	2.30	2.07	1.58	2.72
H_2O	4.95	3.22					
CO_2				6.10	3.46	8.45	3.68
TiO_2	.49	.52					
P_2O_5	.17	.15			.20		
MnO	.21	.38					
N	.37	.39		.16			.16
Ignition	3.61	5.62	14.60	6.73	8.19	5.14	13.44
Total	99.42	101.28	99.98	100.00	99.73	99.00	100.00

[a] A.—Dustfall at Madison, Feb. 13, 1923.
B.—Dustfall at Madison, Mar. 19, 1920 (*12, p. 849*).
C.—Dustfall at Otakaia, New Zealand, Nov. 14, 1902, which came about 1,500 miles from Australia (*6*).
D.—Dust in "red rain" fall at Lamberhurst, England, Feb. 22, 1903; 2.19 per cent of organic carbon included in total (*10, p. 54*).
E.—Dustfall at Naples, Italy, Mar. 10, 1901. (*7, p. 157.*)
F.—Dustfall at Naples, Italy, Feb. 25, 1879, analysis by Scacchi, quoted by Palmeri (*7, p. 161*); 4.16 per cent of organic material and 1.39 per cent "loss" included in total.
G.—Dustfall at Taormina, Sicily, Mar. 19, 1901. The fall amounted to 5½ tons per square mile (*10, p. 222*); 9.89 per cent of organic carbon and 0.32 per cent of CoO included in total.

TABLE V.—*Chemical composition of Mississippi Valley loess compared with that of Madison dustfalls*

Constituents	Percentages							
	A [a]	B	C	D	E	F	G	H
SiO_2	79.77	74.46	72.68	71.73	70.86	70.11	67.20	64.61
Al_2O_3	9.95	12.26	12.03	7.85	8.91	14.25	13.71	10.64
Fe_2O_3	3.39	3.25	3.53	3.93	2.97	4.02	2.17	2.61
FeO		.12	.96		.10			.51
MgO	.26	1.12	1.11	1.00	3.12	1.32	1.76	3.69
CaO	.67	1.69	1.59	1.83	4.13	1.53	1.74	5.41
Na_2O	1.08	1.43	1.68	1.17	1.69	1.09	2.11	1.35
K_2O	2.05	1.83	2.13	2.11	1.18	2.03	2.30	2.06
H_2O	2.55	2.70	2.50	4.95	1.10	2.48	3.22	2.05
CO_2		.49	.39		4.70			6.31
TiO_2	.70	.14	.72	.49	.59		.52	.40
P_2O_5		.09	.23	.17	.40		.15	.06
MnO		.02	.06	.21	.28		.38	.05
Total	100.42	99.83	100.22	99.42	99.98	100.33	101.28	100.06

[a] A.—Loess, Terre Haute, Ind. (*8, p. 562*).
B.—Loess, Kansas City, Mo. (*1, p. 282*). 0.12 organic carbon, 0.06 SO_3 and 0.05 Cl included in total.
C.—Loess, Dubuque, Iowa (*1, p. 282*). 0.51 SO_3, 0.09 organic carbon, and 0.01 Cl included in total.
D.—Dustfall at Madison, Wis., Feb. 13, 1923. 3.61 organic carbon and CO_2 and 0.37 N included in total.
E.—Loess, Mt. Vernon, Iowa (*4*).
F.—Loess, Kansas City, Mo. (*5, p. 94*). 3.50 ignition loss included in total.
G.—Dustfall at Madison, Wis., March 19, 1920 (*12, p. 849*). 5.62 ignition loss and 0.39 N included in total.
H.—Loess, Galena, Ill. (*1, p. 282*). 0.13 organic carbon, 0.11 SO_3 and 0.07 Cl included in total.

The dustfall at Madison on February 13, 1923, is the most siliceous on record, although some dustfalls in Europe derived from the Sahara desert are said to reach a maximum of 73.45 per cent of silica.

European dustfalls are often very red, producing the so-called "red rain," or "rain of blood." This is due to complete oxidation of the iron, and is in marked contrast to the incomplete oxidation and gray color of the

Madison dustfalls. The latter also differ from the European dustfalls in the relative scarcity of calcium carbonate. The only foreign dustfall which resembles the American material in this respect is that of New Zealand (Table IV, C.)

In chemical composition the Madison dustfalls resemble the loess of the Mississippi Valley more closely than the dust from the African desert. This is shown in Table V.

The loess from Mount Vernon, Iowa (Table V, E), and from Galena, Ill. (*H*), contains considerably more lime carbonate than the Madison dustfalls (D, G). But in other respects the loess and the dust are strikingly similar. The dust of the 1923 fall (D) is more like the loess in chemical composition than is the dust of the 1920 fall (G).

MINERAL COMPOSITION

It is impossible to determine microscopically the mineral components which form the finest "clay" particles of the dust, but those particles of the size of "very fine sand" (0.05 to 0.10 mm.) can be recognized with little difficulty. In the dustfall of 1923 such particles form one-third of the whole dust, by weight, and therefore give an approximate idea of the mineral composition of the whole, since the particles of different sizes are not radically different in composition. A microscopic examination of the "very fine sand" disclosed the presence of very abundant quartz grains, a few feldspar grains, rather common limonite aggregates and grains thoroughly stained with limonite (which are hardly distinguishable from brown hydrocarbon fragments), and very minor amounts of hornblende, chlorite, carbonate, garnet, zircon, epidote, zoisite, apatite, tourmaline, hematite and magnetite. The feldspar is chiefly microcline with some albite. Most of the material is clear and readily identified except for that which is stained by limonite.

It is possible to calculate the approximate mineral composition from the gross chemical composition by making certain assumptions similar to those made in calculating the mineral composition of igneous rocks. It is assumed that all the soda is in albite feldspar, all the potassium is in orthoclase (or microcline) feldspar, all the phosphoric acid is in apatite, all the titanic acid is in ilmenite, all the magnesia is in chlorite (in case of insufficient alumina the magnesia may be assigned to enstatite), all the lime remaining after forming apatite and calcite is in anorthite feldspar, all the alumina remaining after forming feldspars and chlorite is in kaolinite, and all the silica remaining after forming all these silicates is in quartz. These assumptions are not all true, but they represent approximations and possible (even if not actual) combinations of the oxides into minerals. They furnish a useful means of comparing analyses, especially when the underlying assumptions are based so far as possible on determination of mineral composition by microscopic study. The results of such computations so far as they relate to dustfalls, are given in Table VI.

Table VI shows even more plainly than Table IV that the Madison dustfalls are exceptionally rich in quartz; they contain about as much feldspar as the New Zealand and one of the Naples dustfalls. The tenor of calcite and hematite is much lower than in the European dusts.

Table VII shows the degree of similarity between the mineral composition of the Madison dustfalls and that of loess of the Mississippi Valley.

It is evident that while the Madison dustfalls contain two to four times as much free silica (quartz) as foreign dustfalls they contain about the same tenor as found in loess of the upper Mississippi Valley. The 1920 dustfall contained more feldspar and less kaolin than the loess samples, but these differences do not recur in the 1923 dustfall. Some samples of loess contain abundant carbonate, but others contain little or none, like the dustfalls.

Dr. J. G. Dickson, of the Department of Plant Pathology of the University of Wisconsin, collected and examined five samples of dust-bearing snow, to determine the number of plant disease spores present. The samples were obtained in several locations 4 miles south and west of Madison, Wis., on the bench land. A layer of snow-bearing dust was scraped up without taking the bulk of snow either above or below. This was melted, and 10 cc. centrifuged. Spores were counted by means of a haemacytometer. The results are given in Table VIII.

TABLE VI.—*Calculated mineral composition of American and European dustfalls*

Constituents	Percentages						
	A[a]	B	C	D	E	F	G
Quartz	52.27	36.47	18.36	13.34	5.75	17.32	----
Albite	9.91	17.89	14.21	9.82	22.14	14.04	21.98
Orthoclase	12.50	13.69	15.32	13.64	12.28	9.38	16.15
Anorthite	8.01	7.64	4.73	4.38	4.44	16.17	7.75
Kaolinite	.43	7.94	26.20	28.85	25.22	----	13.01
Chlorite	2.77	9.26	4.20	5.13	8.66	----	6.11
Enstatite	----	----	----	----	----	2.30	----
Calcite	----	----	----	13.86	7.85	19.17	8.36
Apatite	.39	.35	----	----	.46	----	----
Anhydrite	----	----	----	----	2.33	----	----
Hematite	3.70	----	6.54	6.57	7.03	9.19	6.40
Ilmenite	.93	1.01	----	----	----	----	----
Water, etc	8.51	7.03	10.42	4.41	4.41	11.43	20.89
Total	99.42	101.28	99.98	100.00	100.57	99.00	100.65

[a] A—Dustfall at Madison, Wis., Feb. 13, 1923.
B—Dustfall at Madison, Wis., Mar. 19, 1920.
C—Dustfall at Otakaia, New Zealand, Nov. 14, 1902.
D—Dust in "red rain" at Lamberhurst, England, Feb. 22, 1903.
E—Dustfall at Naples, Italy, Mar. 1, 1901.
F—Dustfall at Naples, Italy, Feb. 25, 1879.
G—Dustfall at Taormina, Sicily, Mar. 10, 1901.

TABLE VII.—*Calculated mineral composition of Madison dustfalls compared with that of the Mississippi Valley loess*

Constituents	Percentages							
	A[a]	B	C	D	E	F	G	H
Quartz	58.58	52.27	49.50	48.89	45.56	43.52	39.66	36.47
Albite	9.14	9.91	12.10	14.30	14.22	9.24	11.42	17.89
Orthoclase	12.16	12.50	10.86	6.98	12.62	12.03	12.20	13.89
Anorthite	3.34	8.01	4.74	----	2.19	7.60	----	7.64
Kaolinite	11.55	.43	14.12	12.29	14.83	17.15	15.62	7.94
Chlorite	.71	2.77	3.10	----	1.55	3.65	----	9.26
Enstatite	----	----	----	3.17	1.37	----	3.24	----
Calcite	----	----	1.11	6.43	.88	----	9.39	----
Magnesite	----	----	----	3.56	----	----	4.57	----
Apatite	----	.39	.21	.94	.53	----	.14	.35
Gypsum	----	----	----	----	1.10	----	.22	----
Ilmenite	1.33	.93	.26	1.12	1.37	----	.76	1.01
Hematite	2.76	3.70	3.25	2.97	2.71	4.02	2.17	----
Magnetite	----	----	----	----	1.19	----	.64	----
Water, etc	.85	8.51	.58	----	.10	3.12	----	7.03
Total	100.42	99.42	99.83	100.65	100.22	100.33	100.03	101.28

[a] A—Loess, Terre Haute, Ind. (*8, p. 562*).
B—Dustfall at Madison, Wis., Feb. 13, 1923.
C—Loess, Kansas City, Mo. (*1, p. 282*).
D—Loess, Mt. Vernon, Iowa (*4, p. 189*).
E—Loess, Dubuque, Iowa (*1, p. 282*).
F—Loess, Kansas City, Mo. (*5, p. 94*).
G—Loess, Galena, Ill. (*1, p. 282*).
H—Dustfall at Madison, Wis., March 19, 1920 (*12 p. 349*).

TABLE VIII.—*Number of spores per cubic centimeter of melted snow*

A. SAMPLE NO. 1

Kind of spore	Number of determinations						
	1	2	3	4	5	6	Average
Alternaria sp	0.22	0	0	0	0	0	0.04
Fusarium sp	0	0	0	0	.22	0	.04
Helminthosporium sp	.22	.44	0	.22	0	.66	.26
Rusts, urediniospores	.22	.22	0	.22	0	.44	.19
Rusts, teliospores	0	0	0	0	0	0	0
Smuts	0	.22	.22	0	0	0	.07
Miscellaneous spores	.44	.88	.44	.44	.22	.88	.55
Mycelium	.66	.44	.66	.44	.44	1.10	.62

B. SAMPLE NO. 2

Alternaria sp	0	0	0.22	0	0.22	-------	0.09
Helminthosporium sp	0	0	.22	0	.66	-------	.18
Rusts, urediniospores	0	0	.44	.44	.22	-------	.22
Rusts, teliospores	0	0	0	0	0	-------	0
Miscellaneous spores	.44	.88	1.10	.44	.88	-------	.75
Mscellaneous mycelium	.44	.44	.66	.44	0	-------	.20

C. SAMPLE NO. 3

Kind of spore	Number of determinations					
	1	2	3	4	5	Average
Rusts, urediniospores	0.22	0	0	0	0	0.04
Miscellaneous spores	1.54	.88	.88	.66	1.32	1.06
Miscellaneous mycelium	.88	.44	0	.44	.44	.44

D. SAMPLE NO. 4[a]

Alternaria sp	0	0.22	0	0	0	0.04
Helminthosporium sp	.22	.22	0	0	0	.09
Rusts, urediniospores	.22	0	0	0	0	.04
Rusts, teliospores	0	0	0	.22	0	.04
Miscellaneous spores	.22	0	0	.22	.22	.13
Mycelium	0	.44	.22	0	0	.13

E. SAMPLE NO. 5[a]

Alternaria sp	0	0	0	0	0.22	0.04
Helminthosporium sp	0	0	0	0	0	0
Rusts, urediniospores	0	0	0	0	0	0
Rusts, teliospores	.22	0	0	0	0	.04
Miscellaneous spores	.44	0	.44	.44	0	.27
Mycelium	0	0	0	0	0	0

[a] Samples 4 and 5 contained too many mineral particles to count directly, therefore the melted snow was allowed to stand until the heavy particles settled out, when the counts were made on the decanted solution.

SUMMARY AND CONCLUSIONS

The present paper describes and discusses the third of four dustfalls known to have occurred in this country within the past six years. Two dustfalls have already been reported by the writers, (*11*, *12*), and analyses are under way for a report upon the fourth. The physical, chemical, mineral, and vegetal composition of the dustfall of February 13, 1923, is discussed in connection with that of other dustfalls of this country and abroad, and tabular analyses are presented.

Dustfalls are comparatively rare, and have always occurred in association with storms of late winter and early spring marked by unusually violent winds in the arid and semiarid regions of the plains, the Rocky Mountains, and the southern plateau of the United States.

The close similarity of composition of atmospheric dust to loess, and the divergence of such dust from other types of soils, suggest very strongly that the two are identical. The dustfalls of 1918 and 1920 showed on chemical analysis a higher content of soluble alkalies than is found in loess. Inasmush as the dust-bringing storms came in those instances from the arid southwest, it may be that they furnish the primitive material from which the loess has been formed. In 1923 the scene of deflating winds was closer at hand, and the coarser composition indicates that the atmospheric dust had been transported a slighter distance, perhaps from the region of the Missouri valley, where loess deposits are deepest.

The great depth of the deposits of loess, amounting to hundreds of feet in some places in the States of the Missouri Valley, compared with the present slow rate of deposition of atmospheric dust, indicates that dust-bearing storms were probably more frequent in the period when the loess was laid down. That could have been accomplished if the weather of the glacial period had been more continuously like our present February and March weather. At the same time there must have been extensive areas in the Southwest, uncovered by vegetation, from which the finer soil particles could be blown away by the wind. The fine size of the loess components indicates that they had been carried long distances by the wind, rather than obtained by deflation in the vicinity of deposit.

The presence of viable spores of plant disease in association with atmospheric dust which has been transported hundreds and even thousands of miles, indicates that such diseases may be rapidly spread over longer distances than plant pathologists have hitherto considered possible.

A peculiar psychological phenomenon associated with dustfalls is the fact that scarcely anyone notices them. Inquiries broadcasted during and after a dustfall elicit surprisingly few positive responses. But the complete study of such widespread phenomena as dustfalls demands more extensive attention. The writers will be glad to have the cooperation of other investigators in this field.

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PREPARASITIC STAGES IN THE LIFE HISTORY OF THE CATTLE HOOKWORM (BUSTOMUM PHLEBOTOMUM)[1]

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INTRODUCTION

The occurrence of hookworm infestation in cattle in the United States attracted considerable attention several years ago because of the supposed etiological relation of these parasites (*Bustomum phlebotomum*) to a disease of cattle commonly known as "salt sick." Following the report of Stiles (*14*),[2] who called attention to the occurrence of hookworms in cattle in Texas, Dawson (*5*) pointed out the resemblance between the clinical picture of hookworm anemia in man and "salt sick" in cattle, and in 1906 the same writer expressed a more definite opinion regarding the relation of cattle hookworms to "salt sick," which he characterized as "an acute or chronic parasitic disease manifested at first by low fever, diarrhea, loss of appetite; soon becoming chronic, with continuance of low fever, constipation, loss of appetite, progressive emaciation and pronounced anemia, which in many cases terminates fatally." While it has not been conclusively established that the condition in cattle commonly known as "salt sick" is actually due to hookworm infestation, the fact that cattle heavily infested with these parasites develop a progressive anemia, as shown by Dawson (*6*), has been confirmed by other observers, notably by Reisinger (*13*).

With a single exception, the published reports dealing with hookworm disease in cattle contain no information based on original investigations concerning the life history of *Bustomum phlebotomum*. This exception is a paper by Conradi and Barnett (*4*) which contains a very brief account of the development of the egg up to the infective stage, but lacks essential details. Since precise information concerning the preparasitic stages in the life history of strongyle parasites is generally useful in connection with control measures against these parasites, it is important that pertinent facts concerning the life cycle of cattle hookworms become available in order that such control measures may be devised. Hence the observations and experiments described in this paper were undertaken. As the work progressed some observations and experiments of a less practical nature were included in order to compare the behavior of cattle hookworm larvæ with those of other strongyle parasites.

MATERIAL AND METHODS OF INVESTIGATION

Specimens of hookworms from cattle, collected in the course of post-mortem examinations of the viscera, were washed several times in physiological salt solutions and then chopped with a pair of fine scissors to liberate the eggs from the uteri of the females. With the aid of a pipette chopped-up worm material was added to the surface of feces and charcoal mixtures in glass dishes and bottles (solid culture media) and to water and filtrate from boiled feces in staining jars and Petri dishes (liquid culture media).

The solid culture media were made up as follows: Sheep or cattle feces were boiled in water and filtered through ordinary filter paper. Sufficient charcoal was added to the filtrate to make a thick paste, which was spread out in Petri dishes, wide-mouth bottles, and other glass containers, care being taken to smooth down the surface of the medium and to keep the latter moist by adding water whenever necessary in order to make up the loss due to evaporation. When diluted with water sufficient to make a transparent medium, the filtrate from the boiled feces was found to be an excellent culture medium for the examination of contents from day to day. A thin layer of the liquid was added to the glass containers, thus affording a supply of oxygen, which is requisite to rapid development. Culture media were kept at room temperature (70°

[1] Received for publication Apr. 10, 1924—issued January, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 458.

to 80° F.) and examinations were made by mounting the glass vessels containing liquid media directly on the stage of the microscope for general observation, and resorting to isolation of larvæ whenever detailed observations on structure or behavior were desirable.

Inasmuch as not all eggs hatch at the same time, because of variation in rate of development and various other factors, newly hatched larvæ were isolated and mounted on cover glasses inverted over hollow ground slides or over ordinary slides to which temporary chambers were sealed. Thus the changes undergone by the larvæ could be followed very readily without such confusion as would result from having larvæ at various stages of development in the same dish. If precautions are taken to secure good-sized drops, and if hanging-drop preparations are properly sealed, the changes in the larvæ from the time of hatching to the infective stage may be followed without adding additional fluid, thus avoiding the need of removing the cover glass at various intervals. In one series of observations eggs and larvæ were secured from day to day from solid culture media and their development was compared with that of eggs and larvæ in liquid media. No noteworthy differences in rapidity of development were found; therefore observation on the rapidity of development in subsequent series of cultures was limited to those made on liquid media.

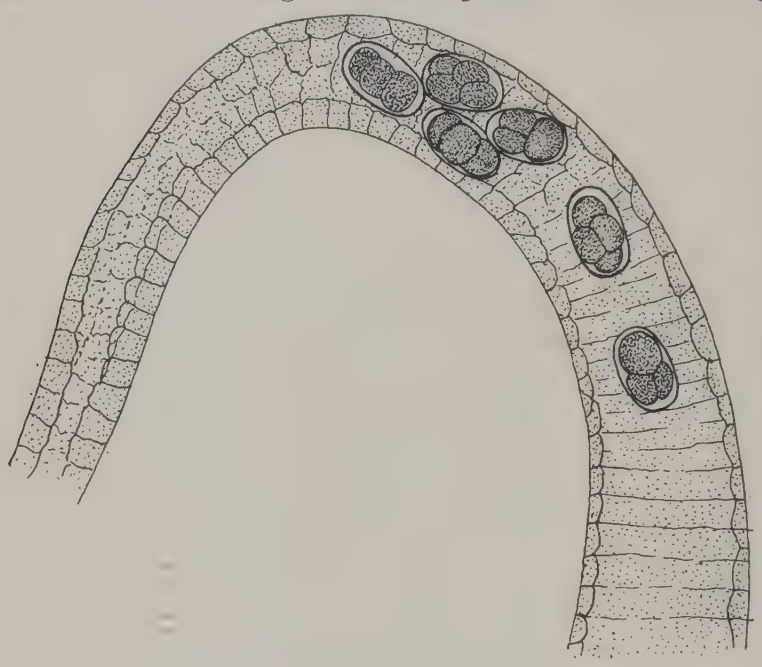

FIG. 1.—Portion of uterus containing segmented eggs

DESCRIPTION OF THE EGG

The egg of *Bustomum phlebotomum*, in common with eggs of other strongyles, is thin-shelled, elliptical in shape, and exhibits considerable range in size (52 to 106 μ in length, by 43 to 60 μ in width). In a series of measurements involving more than 100 eggs only 10 per cent of the eggs exceeded a length of 100 μ and only about 5 per cent were shorter than 75 μ. The majority of eggs measured were from 85 to 95 μ long and slightly over 50 μ wide. No definite correlation was found between length and width of eggs. Some long eggs are comparatively narrow and some present an almost spherical appearance.

DEVELOPMENT OF THE EGG

Conradi and Barnett (*4*) state that before the egg is passed from the body of the host it is in the mulberry stage. These writers also note that partial incubation of the egg in the intestine of the host seems to be important, since eggs that were taken from the host's intestine and thus missed the partial incubation period in the intestine showed a heavier mortality than eggs collected from feces. The present writer noted that eggs begin to segment in the uterus of the female parasite, as shown in Figure 1. Eggs obtained as a result of chopping freshly collected worms are in the one-, two-, and four-cell stages, and rarely in the eight-cell stage. At room temperature (70° to 80° F.) the development of such eggs takes place rather slowly. After 24 hours of incubation it was noted that comparatively few eggs had reached the mulberry stage; and after 72 hours of incubation comparatively few embryonated eggs, with embryos moving in the shells, were found. Ninety-six-hour cultures usually showed free larvæ as well as embryonated eggs, the latter hatching in the course of a few hours. According to Conradi and Barnett (*4*), at a temperature of 48° to 60° it required 31 days for the eggs to hatch. These writers also report that eggs obtained from feces on February 26 hatched on February 28; but they do not give the temperature at which the eggs were incubated. According to observations by the present writer, eggs at various stages of development undergo disintegration in culture media not only because of the presence of bacteria in

cultures, but also because of other factors probably present within the egg as well as in the surrounding medium.

PREINFECTIVE LARVÆ

The first-stage larva exhibits lively movements, twisting its body in typical nematode fashion. Several first-stage larvæ were kept under observation about seven hours, during which period they were incessantly active, moving about with vigorous flexures of the anterior portion of the body aided by the propelling movements of the long, slender tail.

These larvæ are commonly from 420 to 450 μ long, or somewhat longer, by 20 to 25 μ wide. The first third of the body is the broadest, the remaining portion tapering gradually and terminating in a slender tail. Structurally, the organism is relatively simple, its most conspicuous organs being an alimentary canal consisting of an esophagus with a terminal bulb and a straight intestine (this being densely granular in contrast to the less granular esophageal region), and a genital primordium.

About 24 hours after hatching, first-stage larvæ became very sluggish, a physiological condition (lethargus) which precedes molting. Examination of such sluggish larvæ with high magnification generally showed a separation of the cuticle in the cephalic extremity, revealing a newly formed cuticle underneath the old one.

The span of life of the second-stage larvæ is comparatively brief, since 18 hours after the first lethargus was observed the second lethargus was found to be in progress. The second-stage larva shows a slight increase in size (490 by 25 μ) and is less coarsely granular than the first-stage larva. Before its quiescent stage preparatory to the final molt it shows moderate activity, less marked, however, than that of the first-stage larva.

Although in a 6-day-old culture several quiescent larvæ undergoing the final molt were found, third-stage larvæ were generally not observed before the eighth day after making the culture. The second lethargus lasts at least 24 hours.

The duration of the preinfective stages in the life of the larvæ may be increased by subjecting them to low temperatures, which retard their development. Thus first-stage larvæ were prevented from further development for a period of 10 days by being kept in water in a refrigerator at a temperature of 8° C. While a number of larvæ degenerated during that period, others were still alive and showed no changes of structure or other evidence of having molted. Meanwhile larvæ from the same lot at room temperature under-

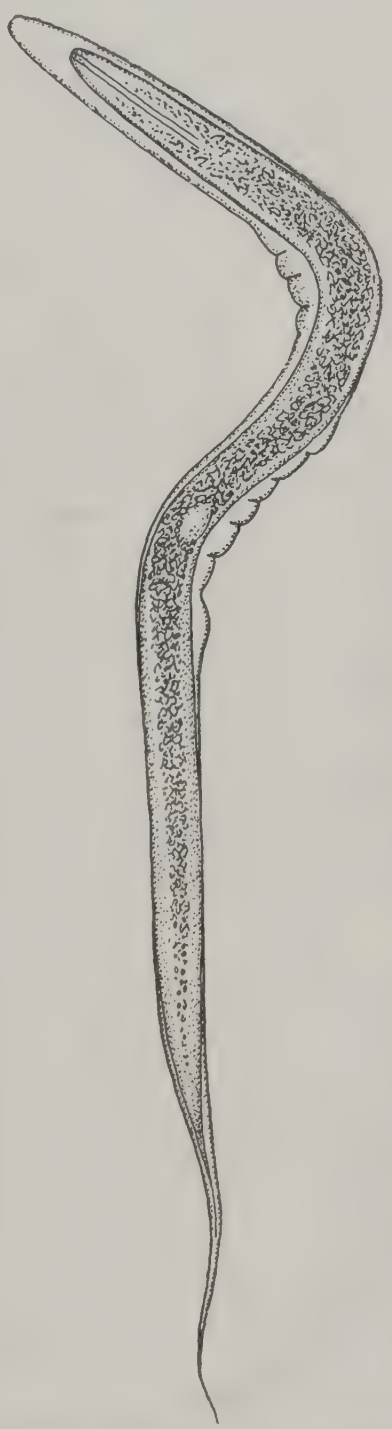

FIG. 2.—Outline drawing of infective larva from solid culture medium showing protective sheath

went their normal course of development.

INFECTIVE LARVÆ

In rich 8-day-old cultures third-stage larvæ were found in abundance. These organisms are readily recognized by their greater transparency, which in-

creases as they are kept alive in cultures. The earlier-stage coarser granules practically disappear and the larvæ also retract within the shells, showing quite distinctly the protective sheath, which is usually wrinkled (fig. 2).

The infective larvæ are generally from 500 to 540 μ long by 20 to 27 μ wide, although infective larvæ slightly less than 500 μ long were found on several occasions. The distance from the cephalic extremity to the base of the esophagus was found to vary from 125 to 145 μ. The diameter of the esophagus at the base is 10 μ. The nerve ring has a diameter of 5 μ and is usually situated 60 μ from the cephalic extremity. In one specimen the distance was found to be 78 μ, while in another specimen it was only 50 μ. The distance from the anterior extremity to the genital primordium is slightly in excess of 200 μ. The genital primordium is 10 μ long by 5 μ wide. The tail corresponds roughly to the terminal sixth of the total body length (fig. 4).

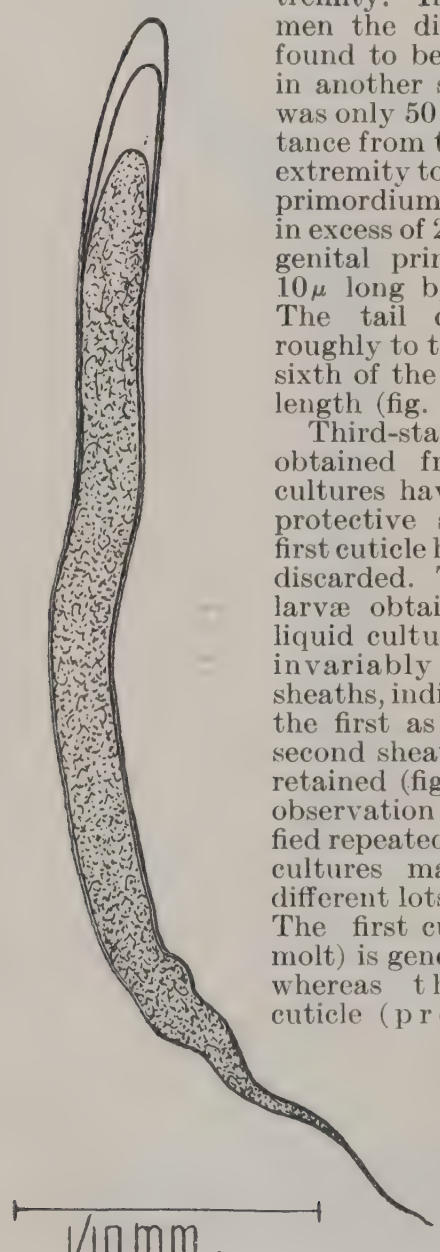

FIG. 3.—Outline drawing of infective larva from liquid culture medium showing two sheaths

Third-stage larvæ obtained from solid cultures have only the protective sheath, the first cuticle having been discarded. Third-stage larvæ obtained from liquid cultures almost invariably show two sheaths, indicating that the first as well as the second sheath has been retained (fig. 3). This observation was verified repeatedly in liquid cultures made from different lots of worms. The first cuticle (first molt) is generally rigid, whereas the second cuticle (protective sheath) is flexible and is often wrinkled.

Larvæ with two sheaths from liquid cultures were kept under observation in cover-glass preparations, and it was noted that as the slide became almost dry certain larvæ that were in contact with some solid object discarded the first sheath. This observation was repeated a number of times and indicates that contact with a solid object is necessary to exsheathing, and also probably that a liquid medium is inimical to the completion of the ecdysis. According to Augustine (1), the first ecdysis in *Ancylostoma duodenale* is due to the fact that the larva has increased in size considerably and thus bursts the tightly surrounding sheath. The slight difference in size between first- and second-stage larvæ of *Bustomum phlebotomum* renders other mechanical stimuli necesary for the completion of she first ecdysis, and in the absence of these stimuli the first cuticle is retained.

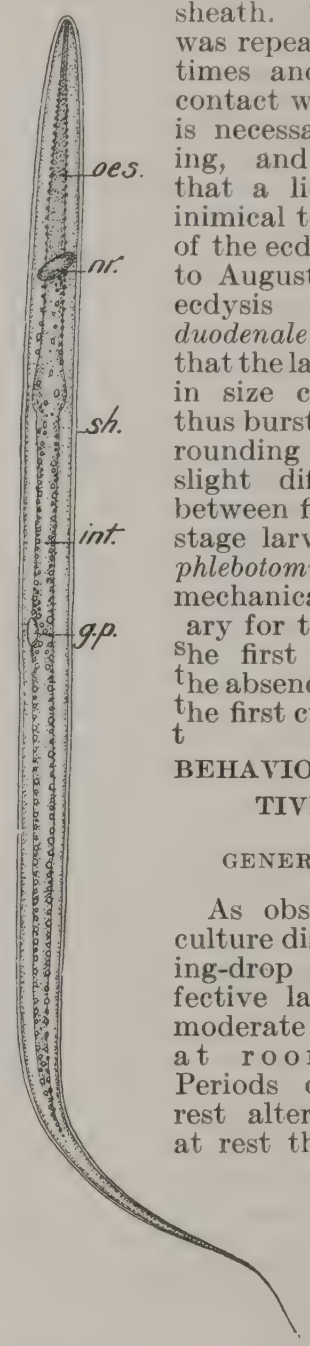

FIG. 4.—Infective larva. oes., oesophagus; n. r., nerve ring; sh., sheath; int., intestine; g. p., genital primordium

BEHAVIOR OF INFECTIVE LARVÆ

GENERAL BEHAVIOR

As observed in liquid-culture dishes and in hanging-drop preparations, infective larvæ show but a moderate degree of activity at room temperature. Periods of activity and rest alternate, and while at rest the larva is either straight and rigid or somewhat coiled and rigid. The protective sheath is generally visible, because the body is always more or less retracted within the sheath. Quiescent third-stage larvæ usually become active when disturbed. A transfer from a culture dish to a slide stimulates them to activity involving rapid movements, which gradually become less intense and ultimately cease entirely.

On slide preparations it was noted that the larvæ commonly exhibit a tendency to come to rest when in contact with some solid object such as débris carried over from the culture medium. Larvæ with the anterior

portion of the body concealed under some solid object and the rest of the body straight and rigid are usually seen in culture dishes and cover-glass preparations.

Infective larvæ appear generally more tolerant of an unfavorable environment than are preinfective larvæ. Preinfective larvæ are easily killed in culture dishes when bacteria are present in large numbers, the heaviest mortality occurring during the lethargus. But infective larvæ were found in culture media containing massive bacterial growths. While preinfective larvæ can maintain their vitality at a low temperature, they frequently degenerate in a refrigerator, whereas this phenomenon was not observed in infective larvæ, which were kept at 8° C. in water without showing any signs of degeneration. Infective larvæ were also frozen solid for about 15 hours outside of a window, and after being thawed they gradually resumed their normal shape and became active, showing what appeared to be a complete recovery in about 5 hours, whereas preinfective larvæ are easily killed by cold, according to Conradi and Barnett (*4*). Ransom (*12*) showed that the preinfective larvæ of *Haemonchus contortus* offer comparatively little resistance to freezing, although infective larvæ could be frozen and successively thawed out many times.

REACTION TO HEAT

Under the influence of heat the larvæ exhibit very lively movements. Thus, if the point of a needle heated in a flame or the heated end of a glass rod is brought into contact with the undersurface of a glass slide containing larvæ, they become very active and orient themselves toward the source of the heat, moving very rapidly in that direction. The orientation of the larvæ with reference to the source of heat is unfailing, and the writer took advantage of this reaction to concentrate larvæ in the center of a dish in which comparatively few larvæ were present. Holding a hot glass rod against the outer surface of the bottom of a Petri dish caused the larvæ to collect in a circumference around the point to which heat was applied, approaching thither from all directions.

While heat is an unfailing source of stimulation to the larvæ, it was noted that larvæ which collected near the edge of a drop of water on a slide, a tendency which they frequently exhibit, do not orient themselves toward the source of heat but merely exhibit feverish activity by vigorous and spasmodic movements of the body, without turning the cephalic extremity which continues to be in contact with the periphery of the drop.

If too much heat is applied the larvæ cease their movements before they reach the source of heat, coiling up instead and resuming their activities as the heat diminishes.

Khalil (*9*) showed that the infective larvæ of a number of nematodes are positively thermotropic. He obtained positive results with *Ancylostoma duodenale, Necator americanus, Ancylostoma ceylanicum, Strongyloides stercoralis, Galoncus perniciosus,* and *Trichostrongylus douglasi,* and negative results with *Haemonchus contortus.* As *Haemonchus contortus* is not a skin penetrator, Khalil concluded that only skin penetrators are positively thermotropic. Recently Cameron (*3*) showed that *Monodontus trigonocephalus* is positively thermotropic, although the larvæ do not penetrate the skin under experimental conditions identical with those under which *Ancylostoma ceylanicum* penetrates it. As will be shown later, *Bustomum phlebotomum* does not penetrate the skin under experimental conditions, thus affording further evidence that there is no correlation between positive thermotropism and ability of larvæ to penetrate the skin. In this connection it may be of interest to note that the larvæ of Nematodirus are negatively thermotropic according to Cameron's observations (*3*).

REACTION TO STAIN

The reaction of the infective larvæ to stain was studied by allowing a 1 per cent solution of basic fuchsin to run in under cover-glass preparations containing larvæ. As the stain runs in the larvæ are active at first; but that this activity is independent of the stain and is due to the current can be readily shown by running in water under the cover-glass. As the larvæ become enveloped in stain they generally become quiescent but do not absorb the stain. Larvæ were kept under observation for several hours (6 hours in one case and 3 hours in two other cases), but no penetration of the stain into the tissues of the larvæ was observed. The stain readily penetrates the sheath, as is seen distinctly whenever a larva retracts within the sheath, but the writer found no evidence of penetration of the stain beyond that point. The larvæ remained in the stain for hours without loss of vitality. Their vitality while quiescent can be demonstrated (1) by running water

under the cover glass, the current thus set up acting as a stimulant to activity, (2) by applying heat, (3) by watching for spontaneous signs of activity, which usually occur at variable intervals. Throughout this series of observations, involving a number of larvæ, one larva showed evidence of having absorbed some stain, but it had lost its vitality and showed no response to stimulation.

Cameron (*3*) states that the larvæ of *Monodontus trigonocephalus* when treated with fuchsin absorb the stain rapidly and die, in contrast to the behavior of the larvæ of *Ancylostoma duodenale*, which fail to absorb the stain and escape from their sheaths, which alone become stained, as shown by Looss (*10*) and by other investigators. Cameron also states: "Goodey (*8*) showed that this was also true for Necator larvæ; but that Haemonchus and Graphidium which do not penetrate the skin, absorbed the stain and died." He further states that all skin penetrators that have been tested exsheathed, whereas nonskin penetrators have not exsheathed but died when treated with stain. In Goodey's paper to which Cameron refers the only statements concerning reaction of nematode larvæ to stains are the following: "I found that *N. americanus* larvæ come out of their sheaths, as found by Herman and confirmed by Looss, when the drop containing the larvæ and stain is covered with a cover slip. *G. strigosum* and *T. retortaeformis* larvæ did not exsheath." Nowhere in Goodey's paper are there any references to the behavior of Haemonchus larvæ in the presence of stain or any reference to the fact that the larvæ of Graphidium and Trichostrongylus were killed by stains (methyl green and fuchsin).

In the present writer's experiments Bustomum larvæ did not exsheath in the presence of fuchsin, nor coil up and die. As will be shown later, there is no necessary correlation between the reaction of larvæ to stains and their ability to penetrate skin.

EFFECTS OF DRYING

According to Conradi and Barnett (*4*), infective larvæ of *Bustomum phlebotomum* are resistant to drought, but according to the writer's observations infective larvæ of *Bustomum phlebotomum* are not resistant to drying. Slide preparations with and without cover glasses containing larvæ were allowed to dry at room temperature for periods varying from one to several hours. After being moistened it was found that the larvæ were retracted in their sheaths, and although the retracted larvæ absorbed water, as a result of which they gradually filled out the empty spaces in the cuticle, they did not regain their vitality. These observations were made during the winter months in a steam-heated laboratory having a very low humidity content, and were repeated a number of times with similar results. Although the larvæ are not resistant to drying, they can maintain their vitality for a long time in the presence of a small amount of moisture. Thus a solid-culture medium in a covered Petri dish to which water had not been added for about three weeks and which had the appearance of being dry yielded larvæ many of which had retained their vitality and exhibited lively movements after being moistened.

Nematode larvæ exhibit considerable variation in resistance to drying. Ransom (*12*) showed that the larvæ of *Haemonchus contortus* are highly resistant to drying, and that they can be revived after 35 days' exposure to drying. Looss (*10*) found that the larvæ of *Ancylostoma duodenale* shrivel up and die when the moisture evaporates from their bodies. Boulenger (*2*) showed that Nematodirus larvæ are resistant to drying, and this observation has been confirmed by Cameron (*3*).

Goodey (*8*) confirms Looss's observation for *Necator americanus* and states: "It seems a natural inference to draw therefore that Necator and Ancylostoma seek the protection afforded by penetration into the skin because if they remained outside and became dry they would perish." The same writer found that the larvæ of *Graphidium strigosum* and of *Trichostrongylus retortaeformis* can withstand ordinary desiccation at room temperature for a few days and revive on the addition of water, behaving in this respect like the larvæ of *Haemonchus contortus* as shown by Ransom (*12*) and confirmed by Veglia (*15*). Cameron (*3*) found that the larvæ of *Monodontus trigonocephalus* offer little resistance to drying, and since these larvæ are not skin penetrators, it is unsafe to attempt to correlate susceptibility to drying with the habit of boring through the skin. The present writer's observations with reference to the behavior of the larvæ of *Bustomum phlebotomum*, which do not penetrate the skin under experimental conditions, lend no confirmation to the view that nonskin penetrators are resistant to drying.

REACTION TO LIGHT

The ensheathed larvæ of *Bustomum phlebotomum* are positively phototropic, as the following observations will show.

In a 14-day-old culture on a charcoal-and-feces mixture larvæ were found along the walls of the bottle facing the light of a north window, but none was found along the walls of the bottle facing the room. In a 10-day-old culture which was kept in a dark place no larvæ were found along the walls of the bottle, whereas in a culture made from the same lot of worms but kept near the window larvæ were found crawling up the walls of the bottle facing the light. Similar observations made at various times on larvæ in cultures indicate that they respond positively to light by collecting in parts of culture dishes exposed to light and by being absent from shaded portions.

In their light reactions the larvæ of *Bustomum phlebotomum* resemble the larvæ of Monodontus (Cameron, *3*) and *Haemonchus contortus* (Veglia, *15*).

REACTION TO GRAVITY

Conradi and Barnett (*4*) noted that the larvæ of *Bustomum phlebotomum* crawl up the walls of culture jars. The present writer's observations bear out this point. Whether this upward movement of the larvæ is a negative geotropism or whether it is merely an expression of their positive phototropism is not clear from the data at hand. The tendency of the larvæ to climb up the walls of culture dishes, and their presumably similar tendency to climb up blades of grass and other objects in nature, is doubtless an adaptation to secure an advantageous position in order to complete their life cycle. Ransom (*12*) pointed out the adaptation of the upward migration of Haemonchus and other strongyle larvæ to the feeding habits of ruminants, which favors the ingestion of the larvæ by the host. Similar tendencies have been observed in most larvæ belonging to the superfamily Strongyloidea, Syngamus, according to Ortlepp (*11*), and Monodontus, according to Cameron (*3*), being exceptions.

EXPERIMENTS ON SKIN PENETRATION

In 1919 infective larvæ of *Bustomum phlebotomum* were placed on the skin of guinea pigs, the area on which the larvæ were placed having been previously shaved. In the course of several experiments no local skin reaction was observed in these experimental animals, and intact larvæ were recovered from the skin. Later, experiments were made in accordance with the method devised by Goodey (*7*). Skin from a 7-day-old rat was stretched on a thin sheet of cork in the center of which was a hole about an inch in diameter, and the cork was floated in physiological salt solution in a glass dish kept in an incubator at a temperature of 37°. A drop of water containing a number of larvæ was placed on the skin. Repeated examinations showed the larvæ on the surface of the skin. The larvæ were sucked up in a pipette and examined from time to time on a glass slide. There was no evidence of their having molted. After the experiment had been in progress several hours the drop of water on the rat's skin containing the larvæ was allowed to evaporate. A drop of distilled water was added to the skin and the larvæ were sucked up in a fine pipette and examined microscopically. They were nearly all active. The skin was then fixed in 70 per cent alcohol and cleared in lactophenol. Microscopic examination of the cleared skin failed to show any evidence of penetration by the larvæ.

Cameron (*3*) has shown that the larvæ of a related hookworm (*Monodontus trigonocephalus*) do not penetrate the skin under experimental conditions. Since well-known skin penetrators (Ancylostoma and Necator) penetrate the skin under similar experimental conditions, this method may be considered a reliable test of the ability of nematode larvæ to bore into the skin.

Assuming, therefore, that the results obtained by Cameron (*3*) with the larvæ of Monodontus and the results obtained by the present writer with Bustomum give reliable information as regards the inability of the larvæ of these genera to penetrate the skin, it is evident from the data and discussion presented in the foregoing pages that attempts of various investigators to establish correlations between behavior of nematode larvæ and their probable mode of entry into the host is untenable.

SUMMARY

(1) Under laboratory conditions at a temperature of 70° to 80° F. the eggs of *Bustomum phlebotomum* hatch in about 96 hours. The first-stage larvæ are found in lethargus 24 hours after hatching, and 24 hours later the second lethargus is in progress. After a second lethargus, which lasts at least 24 hours, third-stage larvæ emerge,

the complete cycle of development up to this stage requiring a minimum of 7 days.

(2) In liquid cultures both cuticles are usually retained by the larvæ, whereas in solid cultures the first cuticle is cast off. Observations on the loss of the first skin by infective larvæ from liquid cultures indicate that contact with some solid object is necessary for exsheathing, and that in a liquid medium this process does not ordinarily take place.

(3) Infective larvæ are only moderately active at room temperature, but may be readily stimulated to activity by mechanical factors. These larvæ appear more resistant to an unfavorable environment than do preinfective larvæ.

(4) Infective larvæ are positively thermotropic and orient themselves in such a way that the cephalic extremity becomes directed to the source of heat, toward which the larvæ swim rapidly.

(5) In the presence of a solution of fuchsin the larvæ behave in a manner unlike that observed heretofore in other nematode larvæ. They neither absorb the stain and die nor do they exsheath, but remain alive and apparently unaffected by the stain, which after several hours' contact with the larvæ does not penetrate beyond the sheath or sheaths.

(6) The infective larvæ succumb to desiccation, but can maintain their vitality under conditions which afford a slight amount of moisture.

(7) The infective larvæ are positively phototropic, collecting in the lightest portion of the culture medium. They also crawl up the walls of culture bottles, but whether the latter is to be interpreted as a negative geotropism or is due to the positive phototropism of the larvæ is not certain.

(8) Under experimental conditions the larvæ show no tendency to penetrate the skin.

(9) Attempts made by various writers to correlate the behavior of various strongyle larvæ with their skin-penetrating habits appear to be untenable from the data presented in this paper and from data obtained by others.

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A MYCORRHIZAL FUNGUS IN THE ROOTS OF LEGUMES AND SOME OTHER PLANTS [1]

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INTRODUCTION

During an investigation of a fungus parasite of the roots of peas, it became necessary in the spring of 1922 to examine under the microscope fresh razor sections of rootlets of a great number of plants in different stages of development. In the course of this work it was found that very many of the rootlets of plants which were considered altogether normal were extensively invaded by a characteristic fungus which entered a few rootlets when the plant was small and had traversed at least half of the root system by the time the plant reached full bloom. The fungus was restricted to the primary cortex of the root, and usually indicated its presence by the straw-yellow color that the root assumed. When the pea plant was mature, all of the roots were usually yellow and thoroughly invaded by the fungus. This uniform and widespread infestation of pea roots indicated that the fungus must be very thoroughly distributed in the soil, and suggested the examination of roots of other legumes to learn whether they, too, might be hosts of this fungus. It was found that the roots of clover, alfalfa, sweet pea, and some other legumes were as thoroughly infested by this fungus growth as were those of peas.

The root-inhabiting fungus thus found so abundant in the smaller roots of these common and much studied legumes is so coarse in character, has such distinctive structures, and produces such conspicuous discoloration of a portion of the invaded tissue as seen under the microscope, that it is somewhat surprising to find that its presence is not a matter of common knowledge among botanists. The fungus itself, or very similar fungi, have long been known in the roots of some other plants under the name of mycorrhizal fungi, and recently several studies of these root parasites by botanists and pathologists have added many details to our knowledge of their development and have extended the list of plants in which they occur. But the list appears to contain only two species among the Leguminosae.[2]

Since the fungus is clearly parasitic in character, occurring abundantly in all or nearly all plants of the species infested, and producing in the invaded tissue unmistakable local pathological conditions, it became necessary to distinguish between the results of its invasion and those following the attack of more aggressive parasites which were under investigation. This study, which began with the roots of peas, was soon extended to the roots of other legumes, and has produced evidence suggesting that this fungus infestation may influence profoundly the development of some of our common legumes, at least when they are grown under certain environmental conditions. It is the purpose of this paper to record more fully than in a previous note (*3*)[3] the list of plants in the roots of which this fungus is found, to describe the local effect it produces, and to indicate the modification in the development of some of the infested plants to which this parasitism may contribute.

It may be unnecessary to add that the term mycorrhizal fungus, in its original coinage and as used in some recent papers dealing with this type of parasitism, does not carry with it any implication in advance of proof that the fungus is of any benefit to the plant invaded. In fact, it seems to be generally recognized that among the numerous observed examples of root invasions which have been designated by this term, many will undoubtedly be found to be wholly disadvantageous to the host and are properly within the plant pathologist's field of study.

[1] Received for publication May 28, 1924—issued January, 1925.

[2] Since this was written Peyronel has published a host list that includes many of the common cultivated legumes. Peyronel, B., prime ricerche sulle micorize endotrofiche e sulla micoflora radicicola normale delle fanerogame. Riv. Biol. 5: 463–485, illus., 1923; 6: 17–53, illus., 1924.

[3] Reference is made by number (italic) to "Literature cited," p. 470.

The literature on the type of mycorrhizal relation considered in this paper has been reviewed in recent papers by Magrou, Peyronel, and Demeter, cited elsewhere. It will suffice to note here that the first description of a mycorrhizal fungus in a plant belonging to the Leguminosae appears to have been made by Janse (*2*) in *Pithecolobium montanum*. Magrou (*4*) has given an excellent description of a similar or identical fungus in *Orobus* (*Lathyrus*) *tuberosus*. Doctor Magrou has exchanged stained preparations with the writer, and he states in personal correspondence that the fungus in the roots of the four legumes sent him appears identical with that which he found in *Orobus*. The writer concurs in this opinion. Peyronel (*5*) has recently published an abstract of a forthcoming paper recording this type of mycorrhizal invasion in a number of plants, and he describes what he believes to be the method of germination of the so-called vesicles. Demeter (*1*) has found the same type of mycorrhizal fungus in roots of *Vinca minor* and some related plants. His attempts to secure it in pure culture have yielded the first success that has been reported. From its cultural characters Demeter designates the fungus *Rhizoctonia apocynacearum*, without giving a formal description; but his attempts to infect plants with this culture did not satisfactorily reproduce the condition found in the field. In view of these unsatisfactory inoculation experiments and the obviously great differences between the fungus described in culture and that found in the plant, Demeter's conclusions need confirmation.

DISTRIBUTION OF THE MYCORRHIZAL FUNGUS IN THE ROOTS OF LEGUMINOUS PLANTS

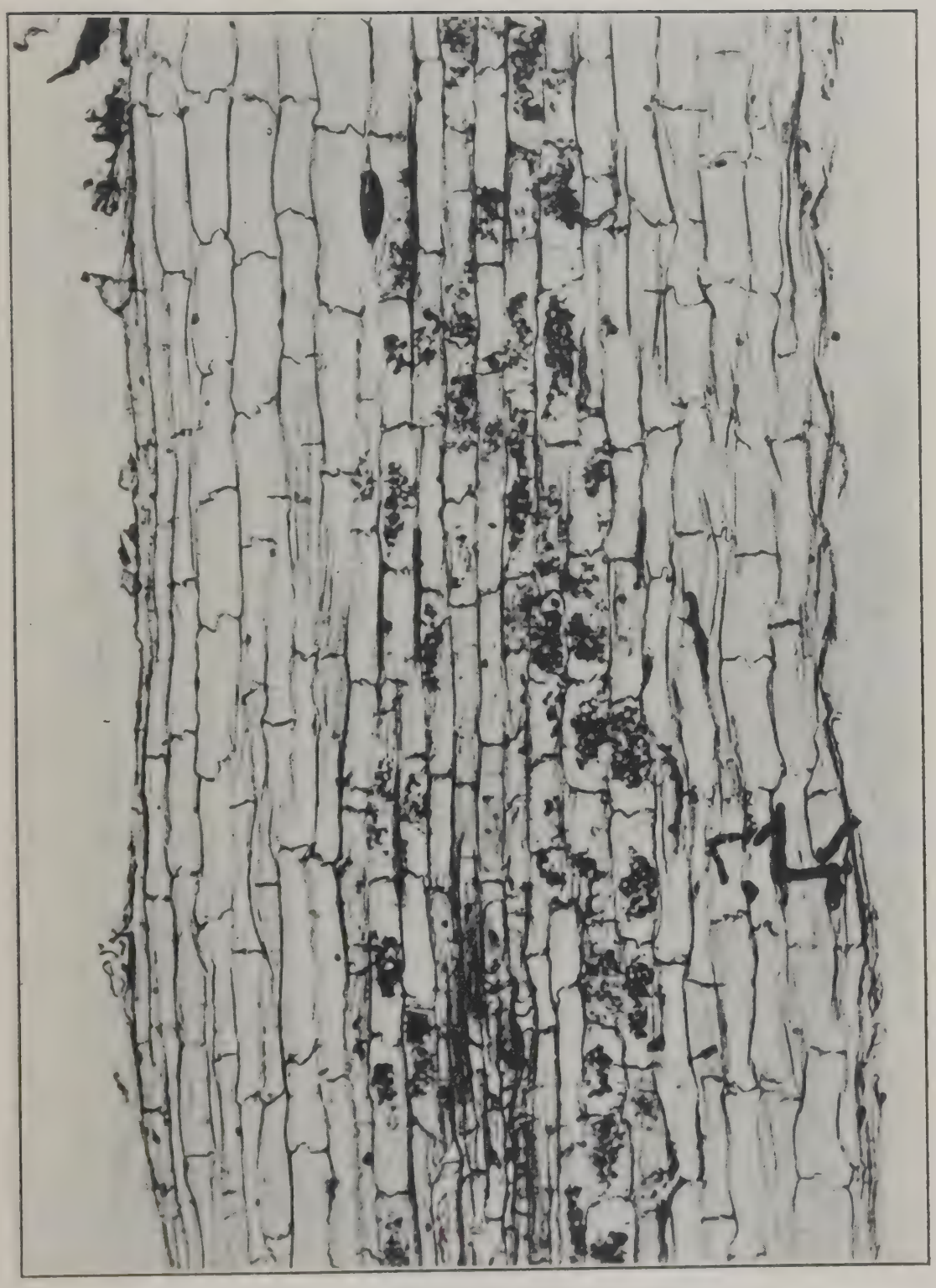
The mycorrhizal fungus of legumes, like that of similar character in other plants, is found only in the cortex of roots. In annuals such as peas and sweet peas it does not enter the cortex of the base of the stem, which is very rootlike in structure. In fact, the cortex of the taproot seems to be quite resistant to it. It develops most abundantly in the small lateral roots which produce little or no secondary thickening. In biennials and perennials such as clover and alfalfa the cortex of the taproot is sloughed off as soon as secondary growth increases the diameter of the root. Therefore the fungus is not commonly found in taproots or even in the large lateral roots. The small laterals and terminal branches of all roots in older plants produce little or no secondary thickening, and the primary cortex persists during the life of the roots. This cortex is readily penetrated and in it the fungus develops extensively. All the above-named plants have a comparatively thick cortex which occupies from two-thirds to three-fourths of the entire diameter, and in these hosts (Pls. 1, 2) the fungus develops far more abundantly than in the roots of beans and soybeans, which have comparatively thin cortical layers. Thus the thickness and character of the tissue outside the endodermis seem to determine the extent to which the plant may harbor the fungus. Perhaps the large intercellular spaces in the cortical tissue, which permit easy passage of the hyphae along the root, is the character to which the fungus has become adapted.

DISCOLORATION OF ROOTS BY THE MYCORRHIZAL FUNGUS

When the cortex of the root has become thoroughly invaded, the condition is usually indicated by a yellow or greenish-yellow discoloration, sometimes accompanied by a slightly water-soaked, translucent appearance. This discoloration is due, as will be shown later, to the color of the contents of the deeper cells, and to this source is due the characteristic appearance distinguishing it from those discolorations which arise from changes in the walls of the outer cells. In advanced stages the walls of the outer cells may turn yellow as the roots begin to disintegrate or from some other cause, whereupon the characteristic appearance is lost. The larger part of the discoloration in the roots of legumes which have been examined seems to be due to this fungus. The color does not indicate the extent to which invasion has progressed; at times it will be found to have advanced far beyond the discolored region. In all doubtful cases, the presence of the fungus can readily be determined by microscopic examination of sections of the root.

PATHOLOGICAL HISTOLOGY

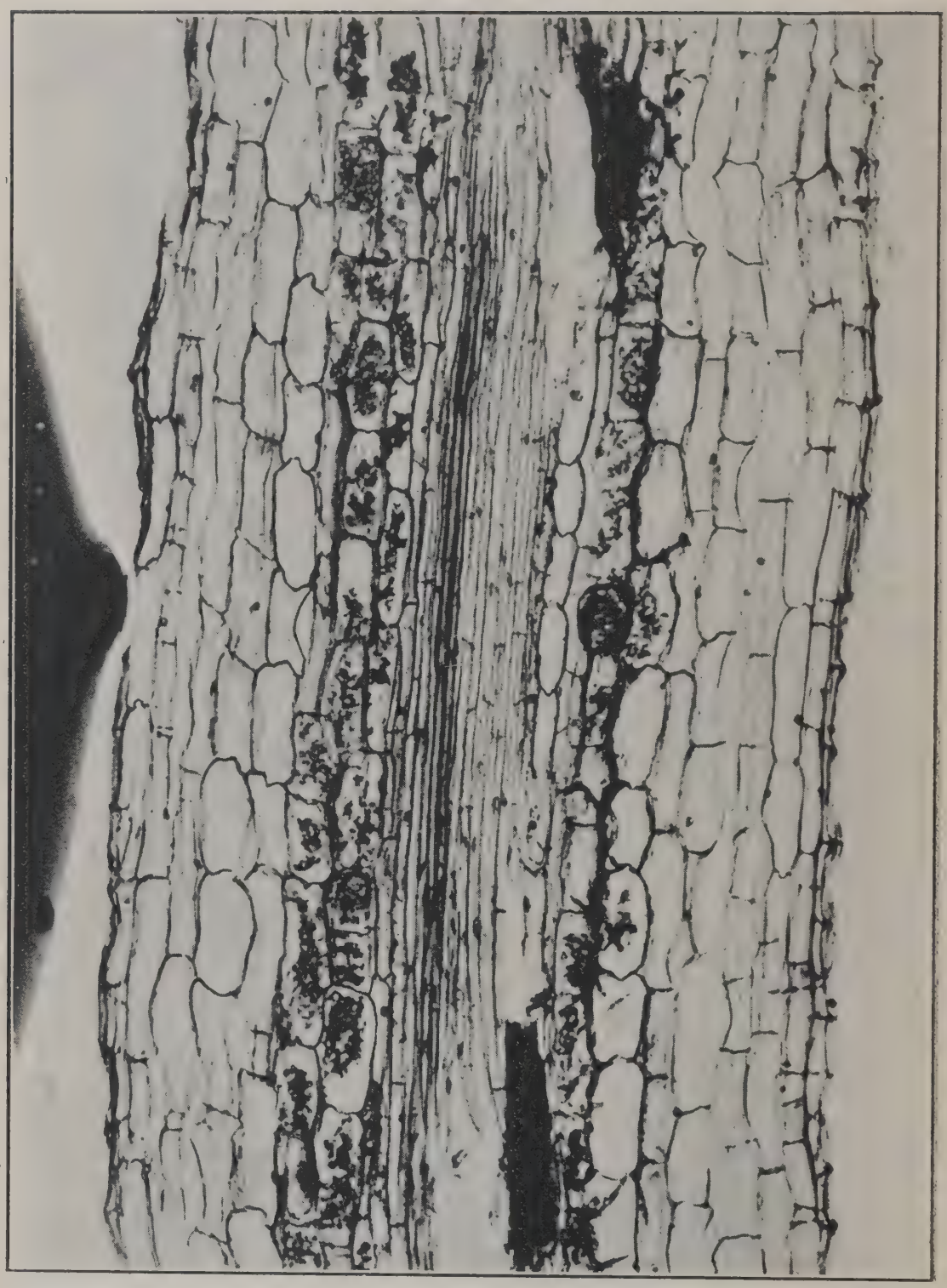
An examination of free-hand longitudinal sections from fresh roots which are yellow and water-soaked will show at once where the color is located. It is usually limited to the cell sap of the cortical cells just outside the endodermis where its greenish-yellow or olive-green character is very striking in thick sections. This color soon diffuses from the cut cells of razor sections mounted in water, and is more slowly lost from



A Mycorrhizal Fungus in the Roots of Legumes

Plate 1

Mycorrhizal fungus in a rootlet of red clover. Longitudinal tangential section including a few endodermal cells at bottom of plate. The penetration of the fungus is shown at the right margin of the root. The dark masses of irregular outline in the cells at the center of the section are arbuscles. A few fragments of longitudinal hyphae are shown between the cells. The dark elliptical body between two cells near the top of the plate is a small vesicle.



A Mycorrhizal Fungus in the Roots of Legumes

Plate 2

Mycorrhizal fungus in a rootlet of red clover. A median longitudina lsection showing the fungus outside of the endodermis. The intercellular mycelium appears as dark lines. A vesicle is shown at the right of the center of the section.

entire roots which are kept submerged in water. The intercellular spaces of the discolored cells are more or less filled with a liquid, this giving rise to which are sometimes even more conspicuous than the color, and these are unmistakable evidence of the presence of the fungus strands, which are some-

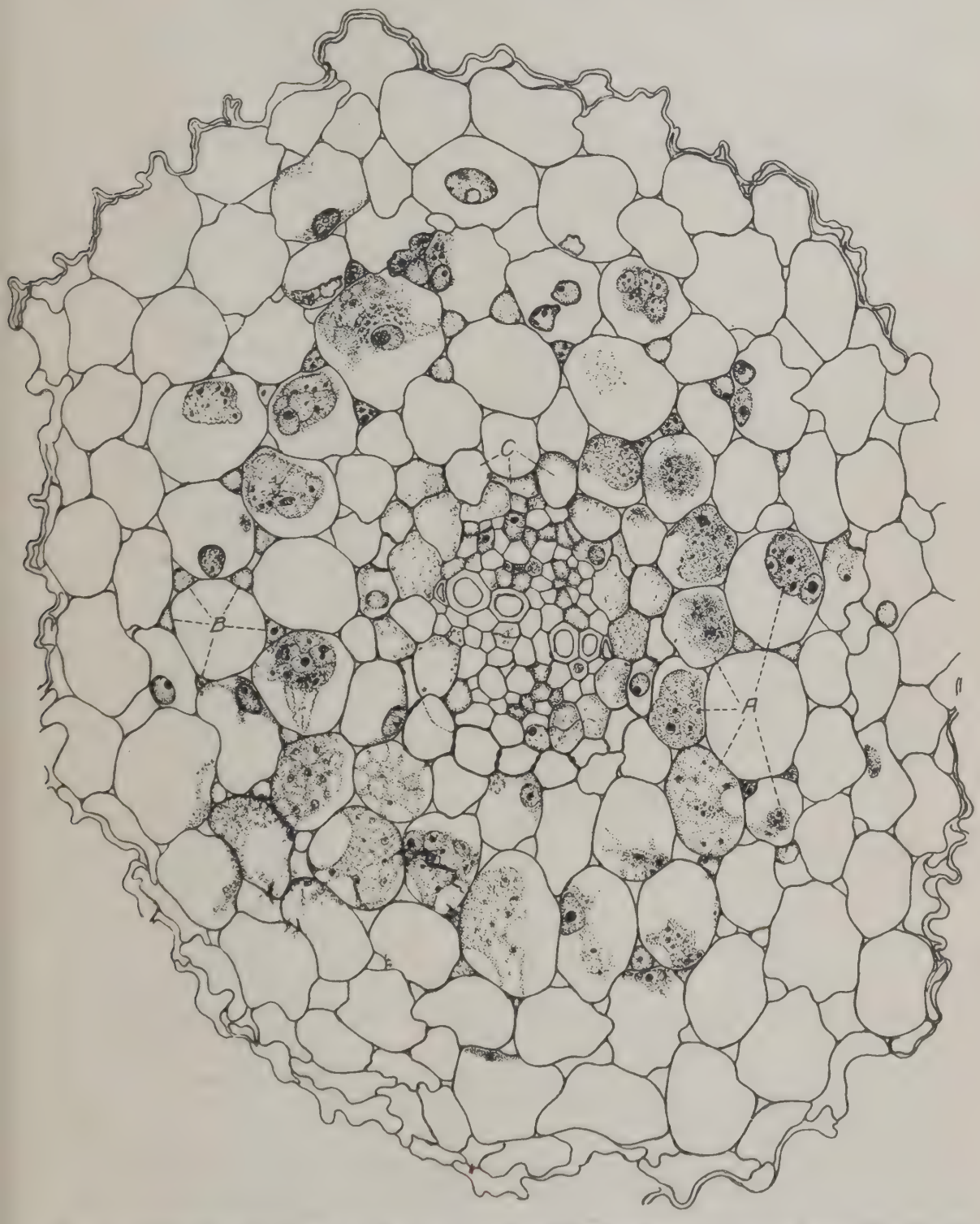


FIG. 1.—Transverse section of a small rootlet of *Trifolium pratense* containing the mycorrhizal fungus. A.—Degenerating haustoria. B.—Strands of intercellular mycelium, of which about 35 may be counted here. C.—Endodermal cells.

the water-soaked appearance of the tissue. The cells with discolored contents usually contain large, somewhat amorphous masses of uncolored material, the haustoria of the fungus (Pl. 1), times difficult to discern in longitudinal sections. A cross section of an infected root shows more clearly the distribution of the strands from which the haustoria originate (fig. 1).[4] Usually they occur

[4] The writer is indebted to Dr. Charles Drechsler for the drawing of all text figures.

only locally in the cortex, but are frequently distributed throughout its radial section. The fact that the tissue is not immediately destroyed indicates that a high degree of adaptation has been established.

DESCRIPTION OF THE FUNGUS

The mycorrhizal fungus in roots of legumes has been described and illustrated so well by Janse, Magrou, and Peyronel that only the more important morphological characters will be described here. The characteristic structures of the fungus have been designated mycelium, arbuscles, and vesicles. The arbuscles are, as Peyronel recognized, haustorial structures, comparable to those produced by some of the Peronosporaceae, though somewhat more elaborate in structure. Since there is no apparent advantage in the use of a distinctive term, they will be called haustoria in this paper. The vesicles have been regarded by those using the term as fruiting structures, probably sporanges, a supposition which is supported by Peyronel's tentative account of their germination. The mycelium is nonseptate, at least within the host. In diameter it varies, depending somewhat upon the character of the tissue in which it grows. The first strands which enter the root are 6 to 11 μ in diameter, whereas those which develop later sometimes measure12 to 13 μ. The hyphal walls are conspicuously thick whether the strands are inside or outside the host tissue. Within the tissue intercellular strands often show small pointed projections extending between the rounded corners of the cells at their junction, suggesting a plasticity of the fungus wall in early development (fig. 2). The comparative thickness of the wall, and the somewhat angular molding of contour at intervals are the more distinctive mycelial characters. The septa, if such they may be called, which are found in mycelium outside the host are usually curved, and appear to be derived from an interior lining of the wall rather than from the thick outer wall.

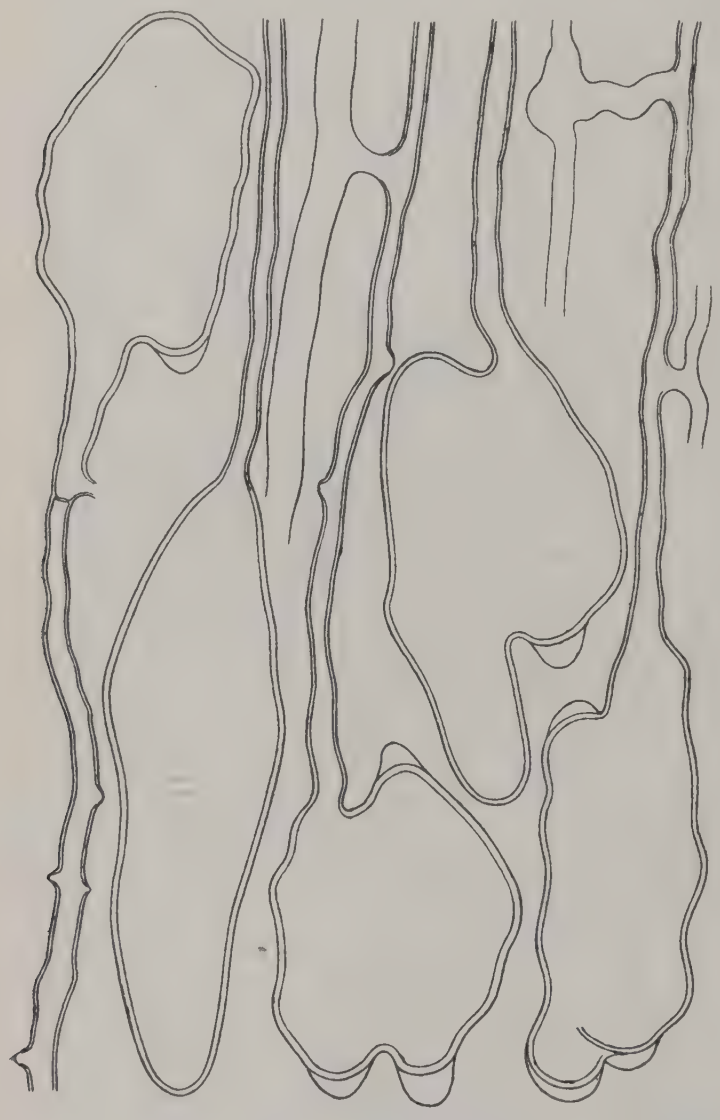


FIG. 2.—"Vesicles" of the mycorrhizal fungus from roots of *Melilotus alba*, showing characteristic shapes which develop from the pressure of surrounding cells.

The haustoria present a complete series of forms from the simplest protuberance of the mycelial wall to branched structures of great complexity. They rarely develop in the outer cells of the cortex in which the mycelium passes readily through cells. In deeper cells haustoria may appear as small protuberances or as thick, blunt projections on intercellular strands. In deeper tissue, which appears to have a richer protoplasmic content, haustoria branch abundantly, producing at the ends of thin hyphal branches a much-lobed, very delicate structure, seemingly without wall or membrane, and often filling a large part of the lumen of the cell (fig. 3). This terminal structure has been interpreted as the product of the digestion of the fungus by the host, and beyond doubt it does disintegrate very soon into an amorphous mass. Whether the haustoria are digested by the tissue which they enter or whether they disintegrate after a brief period of service to the fungus requires further study.

The vesicles are terminal, and usually intercellular in position in the outer cortex, where the mycelium produces but few haustoria. The number produced varies greatly in different roots of the same plant and also in different species. They are usually few in alfalfa and very abundant in sweet clover. In shape they are typically ovate with a slight papilliform projection at the distal end, but pressure from the surrounding cells forces them into a great variety of distorted forms (fig. 1). They vary in length from 75 to 150 μ and in width from 25 to 65 μ. They appear to be separated from the hyphae by merely a thickened portion of the membrane which surrounds the contents of the vesicle. The first vesicles formed in a root usually contain a few large oil globules, and appear imperfectly developed. Occasionally, when the cortical tissue begins to disintegrate, great numbers, which appear filled with small oil globules of uniform size, are formed.

PENETRATION OF THE ROOT BY THE FUNGUS

When small newly infected rootlets are crushed under a cover glass and examined under the microscope, fungus hyphae are often seen passing along the epidermis and entering the root, where the characteristic haustoria develop. The course of penetration has also been traced in stained sections from material imbedded in paraffin. One of these is shown in Plate 1. Figure 3 was drawn from a similar penetration found in a razor section of a sweet-pea root, and shows the characteristic appearance of the fungus in its advance to the deeper cells of the cortex. The hyphal strands outside the root are always empty at this stage. In this instance one of the two entering hyphal branches is greatly swollen in the epidermal cell, producing a structure like an appressorium. The mycelium in the outer cells is empty, and is contorted in a way suggesting that its passage from cell to cell is difficult.

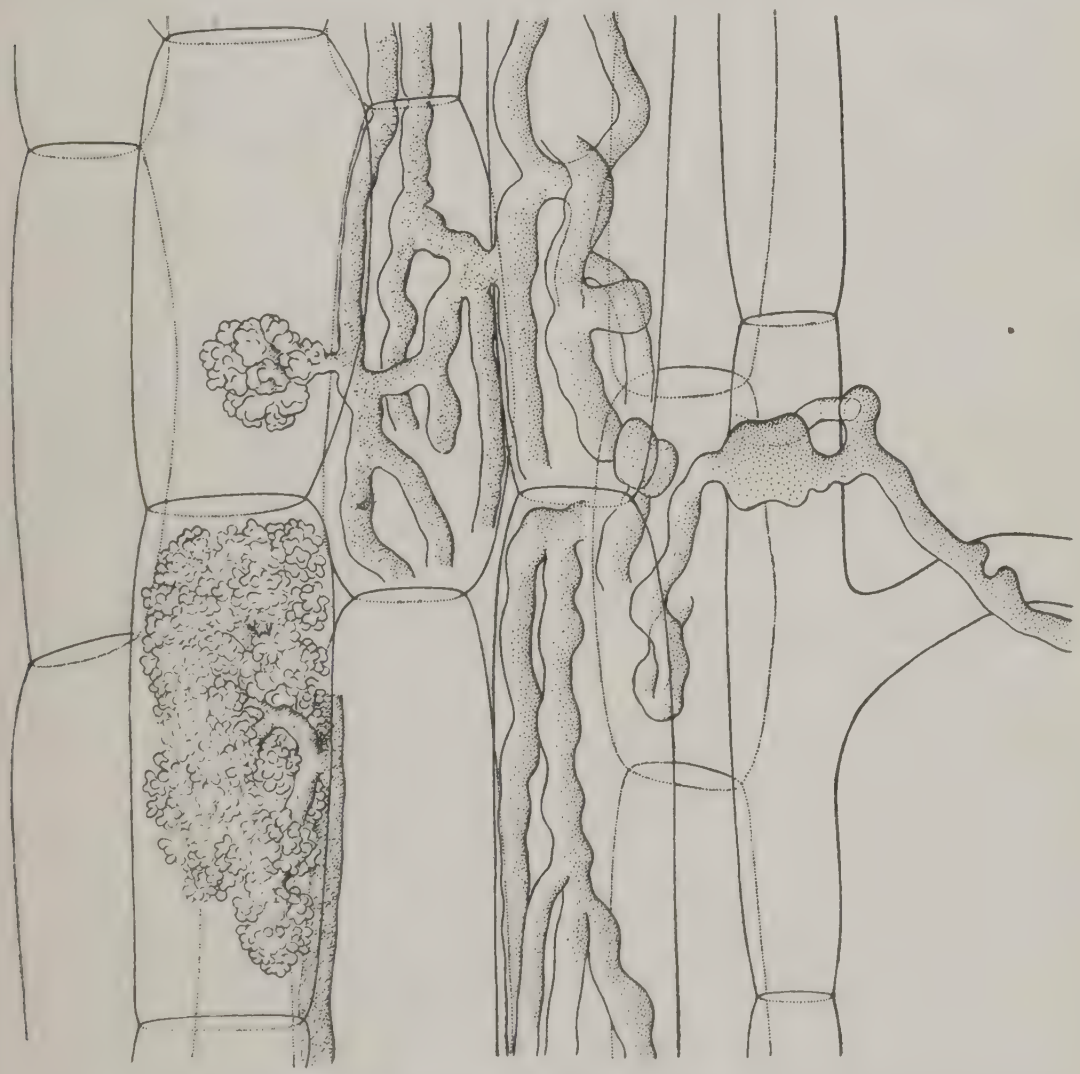


FIG. 3.—Penetration of a root of *Lathyrus odoratus* by the mycorrhizal fungus.

The intercellular strands which produce haustoria have abundant contents. No hyphae filled with protoplasm have been found connecting the mycelium in the root with that outside.

GROWTH OF THE FUNGUS IN CULTURE MEDIA

As soon as the fungus was distinguished by its morphological character in the plant, efforts were made to isolate it in pure culture by the usual laboratory methods. It was found that in the smaller rootlets of alfalfa and clover the fungus was followed so closely by soil-inhabiting fungi and by bacteria that the apparent beginnings of growth of the mycorrhizal fungus were soon submerged and lost. In such rootlets surface sterilization sufficient to destroy the adherent soil organisms always seemed to penetrate the entire cortex. Roots of plants having a thicker cortex, as sweet pea and onion, were then chosen for isolation, and plants were grown in a soil held at a low temperature presumably unfavorable for the troublesome Fusarium species often found in the outer cells. Even in plants thus grown, fungi often grew out from root cortex that appeared uninjured, and bacteria always appeared whenever any fungus growth occurred.

In these isolation experiments the only culture media which appeared to give any growth at all of the mycorrhizal fungus were agar alone or prune agar. When razor sections of roots containing mycelium with abundant protoplasmic contents were thoroughly washed in sterile water, stripped of the outer layers of cortical cells by the careful use of dissecting needles under the low power of the microscope, and placed in poured plates of clear agar before they had solidified, fungus strands which developed could usually be traced to their origin by observation through the bottom of the plate with the microscope. The only fungus strands in such plates which were traced to the mycelium with haustoria in the host cells so clearly that their origin seemed indubitable were of a distinctive character, differentiating them clearly from most fungi in such cultures. These fungus strands were of large diameter, like those in the host tissue, narrowing to a blunt point at the end, and were thrust into the agar almost as straight as stiff bristles, rarely showing a flexuous course or having branches. On the third or fourth day after the plate was made the growth sometimes attained the length of 3 to 4 mm. After this the granular protoplasmic contents began to recede, leaving a series of thin membranous septa. The recession of the protoplasm was usually contemporaneous with the appearance of great numbers of bacteria in the root tissue and its rapid destruction. The agar seemed unable to support growth of the fungus when the host tissue failed. When the longer strands were severed from the host tissue with a sterile needle the protoplasmic contents oozed from the cut ends, and growth ceased. After observation of growth from thin sections seemed to establish the character of the mycorrhizal fungus developing in these culture media, many segments of invaded roots were given surface sterilization with bichloride of mercury and placed in agar, some liquid media, and sterile soil; but in every occasional instance where fungus growth was detected a bacterial growth also occurred, destroying the plant tissue and submerging the fungus. Thus no method of separating the mycorrhizal fungus from its bacterial associates was found, nor did any of the culture media tried appear capable of supporting the fungus growth apart from the host tissue. The fact that strands of the fungus can be found loosely attached to the roots has been regarded as indicating its ability to develop outside of plant tissue, and presumably on artificial substrata; but it is not impossible that this appearance is deceptive, and that the fungus is in fact an obligate parasite capable of growing only in or closely attached to root cortex.

SPECIES OF LEGUMINOSAE IN WHICH THE MYCORRHIZAL FUNGUS HAS BEEN FOUND

During the summer of 1923 the roots of all easily accessible legumes were examined. The fungus was found in the following plants:

Falcata comosa (L.) Kuntze (*Amphicarpa monoica* Ell.).
Astragalus parviflorus (Pursh) MacM.
Lotus americanus (Nutt.) Bisch. (*Hosackia americana* Piper).
Lathyrus odoratus L.
Lathyrus tingitanus L.
Medicago sativa L.
Melilotus alba Desr.
Melilotus officinalis (L.) Lam.
Petalostemum purpureum (Vent.) Rydb.
Phaseolus vulgaris L.
Soja max (L.) Piper.
Trifolium pratense L.
Trifolium hybridum L.
Trifolium repens L.
Vicia sparsifolia Nutt.

There was great variation in the extent to which the root systems were invaded. Roots with a relatively thick cortex, as of peas and clover, generally showed more abundant development of the fungus and a more extensive invasion than beans which have a thinner cortex. There appears to be a difference in infestation between closely related species, the roots of which can hardly be distinguished morphologically. Thus red clover appears to be more thoroughly infested than alsike or white clover.

The fungus was not found in roots of *Oxytropis* sp., *Cicer arietinum* L., or *Lupinus perennis* L. There is no apparent explanation for the complete absence of the fungus from these three species.

DISTRIBUTION OF THE FUNGUS IN AGRICULTURAL LAND

The territory in which the mycorrhizal fungus was first found in native as well as in introduced legumes is comparatively old agricultural land, on the greater part of which leguminous crops have been grown repeatedly. In these older districts many growers believe, not without some good evidence, that certain legumes, especially clover, do not flourish as when first introduced. This opinion is supported by the fact that clover, at least, is at present more successful in newly cleared land. Alfalfa also is not generally so successful in the humid eastern States as in regions of lesser rainfall or under irrigation. These facts suggest the question whether there is any correlation between the abundance of this mycorrhizal fungus and the vigor of these plants. Perhaps newly cleared forest land in which leguminous plants were not numerous and semiarid land with a small plant population of any kind are when first cultivated less thoroughly infested or are free from this fungus.

These questions were in mind when in the summer of 1923 an opportunity was afforded to examine alfalfa and peas in Utah, Idaho, and Montana and also on newly cleared land in Michigan and Wisconsin. It would serve no useful purpose to list here the localities from which specimen plants were taken, or to describe the different cultural conditions under which the plants were grown. It suffices to state that no field however recently reclaimed in arid regions was found free from infestation, and but few mature plants were uninvaded. On newly cleared land in one locality in northern Wisconsin where peas were the first crop, the fungus was found only in plants growing in a few low spots, though at the time examination was made the plants were so young that invasion to a conspicuous degree might not yet have occurred even if the fungus were present. In Montana the fungus was found in the roots of native species on a mountain side far above cultivated fields. Plants in reclaimed swamps in Wisconsin have whiter roots than those in surrounding fields, and indeed in the spring clover has been found in such locations in which no root infestation was discovered. However, a later search in the summer usually disclosed some invasion, though not with accompanying discoloration. It thus appears that not many leguminous plants which are potential hosts reach maturity anywhere in the United States without becoming invaded; and conspicuous vigor in these plants in any locality can hardly be ascribed to the absence of this fungus. On the other hand, it is not easy to determine quantitative differences in invasion in different soils, and much work must be done before such differences will be demonstrated, if they exist, and their effect upon the development of the plants determined.

RELATION OF THE SEASONAL DEVELOPMENT OF THE PLANT TO THE FUNGUS INVASION OF THE ROOTS

During the autumn of 1922 and the entire following year roots of clover and alfalfa were examined from time to time at Madison, Wis., to determine approximately the time at which smaller roots of these plants are developed and when they are invaded by the mycorrhizal fungus. Agronomic and botanical literature appears to contain little information regarding the relation of top growth to root growth in our perennial and biennial legumes, and no information regarding the longevity of the smaller roots. About the middle of April, 1923, as soon as the ground was free from frost, many clover rootlets washed from the soil were found yellow in color. Although the discoloration at this time was largely in the cell walls and had no relation to the presence of the fungus, many of the small roots from all plants in established clover fields contained the fungus, though it was sometimes absent from volunteer plants in fertile gardens. By May 22 the new growth of alfalfa and sweet-clover roots, though varying greatly in different locations, usually amounted to between 1 and 2 inches. In the basal part of this new

growth and extending through about a third of its length the fungus was usually found, apparently advancing from the old root. Plants in soil too dry for vigorous growth showed more invasion than those in more favorable locations. The dry weather, which was also retarding the growth of clover, continued and one week later roots from the most vigorous field were invaded to within an inch of the end, while those from fields a little less vigorous showed in half the roots invasion extending almost to the tip. Soon after this the fungus was found advanced nearly or quite to the root tip in almost all roots, and later in midsummer great numbers of rootlets in the upper foot of soil died. The factors responsible for the death of these roots can hardly be distinguished and evaluated from the evidence at hand. The dryness of the soil alone certainly must have contributed, but it appears also that the invaded rootlets were less resistant to drought, and it may be that the mycorrhizal fungus advanced so far into the growing point of many of them as to cause death. In any case, this loss of roots, as observed during two summers, has left the clover plant with a greatly reduced root system during midsummer. Alfalfa suffers quite as heavily, and during July and August has a surprisingly meager number of living root ends possessing root hairs which can absorb water in the upper 8 inches of soil. Deeper roots do not suffer so great a loss; and in fact the mycorrhizal fungus is less abundant at lower levels, being absent in one instance at a depth of 3 feet. The roots of the plant examined in the instance cited continued to an undetermined depth in very hard subsoil, but there were comparatively few rootlets below the region within which the fungus was abundant.

When rainfall came in late summer and autumn and root development was resumed, longer and longer root ends were free from the fungus. On September 24, the vigorous new roots of red clover near the surface of the soil had 1 or 2 inches of clean growth, while the deeper roots which had grown less rapidly were still invaded almost to their ends. From this time on root growth progressed more rapidly than the fungus invasion. In garden peas, which mature in early or midsummer, there is a steady increase in the proportion of root system invaded until the plant is mature. In the sweet pea, an annual which requires a much longer period of time for its maturity, the root system is found to be thoroughly invaded in mid-July. Little root growth seems to take place thereafter, and the root system slowly dies off during the latter part of the life of the plant.

From this brief outline of the conditions observed in the field it appears that the extent to which roots are found invaded at any time depends largely upon the rapidity with which roots have been growing previously, and presumably also on a temperature favorable to growth of the fungus. Diminished growth of roots at high soil temperatures apparently leads to a complete invasion of the root system. Unfortunately, no study of the rate of root growth of perennial legumes during the season has been made. Therefore much work will be required to determine to what extent root invasion at any time is due to retarded root growth and to what extent it is due to fungus growth.

Some experimental work has been done to determine the range of soil temperature within which the fungus will enter roots. In a preliminary trial in the winter of 1922, it was found that the fungus entered clover roots abundantly at soil temperatures ranging from 16° to 30° C. Another experiment was started in November, 1923, to determine how far beyond this range infection occurs. In this experiment soil was maintained at temperatures of 33°, 15°, 12°, and 9° C. The soil used was loam from woodland, mixed with a small amount of sand containing mycorrhizal-infested sweet-clover roots. Peas and sweet peas were grown in the sand alone. The moisture content of the soil was not determined, but it was ample for good growth, and was maintained uniformly during the experiment. Garden peas, sweet peas, and the taproots of young sweet-clover plants were planted on November 17. Growth of all of these plants was exceedingly slow at 9° C. On December 29, roots of peas at 15° were found extensively invaded, the plants at that time having formed the fifth true node, but no infection was found at 12°. On January 11, peas and sweet peas at 33° had small portions of rootlets invaded, but none of the few branches produced by the Melilotus taproots showed invasion. On January 15 some invasion of sweet pea and Melilotus roots was found at 12°, and finally on February 9, one sweet-pea plant grown at 9° was found abundantly infested. Thus root invasion can take place within the entire range of temperature at which these plants are able to make much growth.

SIGNIFICANCE OF THE MYCORRHIZAL FUNGUS IN LEGUMES

The relation between the fungus and its host plants described in these pages appears to be a long-standing case of mutual adaptation to which the term "symbiosis" may appropriately be applied; but here there is no apparent benefit accruing to the plant invaded. The discoloration and eventual disintegration of the cortex of the roots and the slow growth of the plants at the time when root invasion reaches its maximum suggest that the plant is retarded and injured, at least in so far as vegetative growth is concerned. This may not be determined until the fungus is secured in pure culture. But some evidence of apparent value has already been obtained. This fungus can be destroyed with others in the soil by sterilizing agents, and legumes grown in soil so treated seem to thrive better than in untreated soil. In the spring of 1922, alfalfa, sweet peas, and red, white, and alsike clovers were grown in a small plat which had been treated with formaldehyde the previous autumn, and had been kept covered during the winter to prevent reinfestation. A similar plat was soaked with water to which no formaldehyde had been added. At examinations made during the season, root growth appeared to be about equal for all plants in both plats. But the roots in the treated soil remained white and clean until late in the summer, when a little mycorrhizal infestation began to appear. No other root parasite was detected on plants in either plat. On July 16 the sweet-pea plants in treated soil were twice as tall as in the untreated soil, and had twice as many blossoms. Unfortunately, mosaic appeared in all plants at this time, whereupon blossoming ceased; but the plants in the treated plat remained green throughout the season, while those in untreated soil died and shriveled. The clovers and alfalfa showed little difference until July 30, when the plants in the treated plat stood 1 to 2 inches taller, a superiority which was maintained throughout the autumn. These results agree with Demeter's (*1*) work with *Vinca minor*, but like his are open to the objections that other unrecognized soil parasites may also have been excluded by treatment, and that soil changes favorable to the plant may have resulted from the treatment. Work is now in progress which may furnish more adequate evidence of effect of this fungus on its host plants.

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MYCORRHIZA OF HERBACEOUS PLANTS OTHER THAN LEGUMES

In order to determine whether roots of plants other than Leguminosae are subject to this type of mycorrhizal invasion, a number of cultivated plants and weeds growing at Madison, Wis., have been examined. A similar condition has been found in the following plants:

Allium cepa L.
Asparagus officinalis L.
Aster spp.
Bidens vulgata Greene.
Eupatorium perfoliatum L.
Frageria virginiana Duch.
Ipomoea purpurea (L.) Roth.
Panicun capillare L.
Leontodon taraxacum L. (*Taraxacum officinale* Web.)
Verbascum thapsus L.
Viola sp.
Zea mays L.

A mycorrhizal fungus was not found in a considerable number of other species which are not listed here since the number of plants examined was usually small. It is of interest to record that with the exception of an intensive invasion of rootlets of *Solanum tuberosum* growing in sandy soil in a greenhouse, the author failed to find any mycorrhizal fungus of this type in such solanaceous plants as he has had occasion to examine, this failure being in general agreement with the findings of Magrou (*4*) in France.

None of the plants listed above have been examined in sufficient numbers to indicate clearly whether the fungus is constantly associated with their roots or not. In Panicum and Viola especially, its comparative scarcity in the plants examined suggests that it is not a constant associate. Some varieties of sweet corn seemed more thoroughly infested than other varieties of *Zea mays*, half the small roots in one field being invaded, whereas on the same date (Sept. 1) not more than a quarter of the roots of a dent variety growing close by were found invaded. Roots of strawberry plants from several gardens showed wide differences in extent of invasion. Plants of very vigorous growth from one garden had nearly all rootlets filled almost to the end, while those from a neighboring garden had but few infected rootlets.

Whether the fungus in this wide range of plants belongs to the same or to several species, or to specialized races can not be determined readily until the fungus has been obtained in

pure culture. It may be of interest to add, however, that when onion sets were planted with the legumes in the soil temperature series described previously, their roots became about equally (though more slowly) invaded except in soil at 9° C. This fact may indicate that the same fungus entered both plants, since the soil used was taken from a locality where no species of Allium has been found.

SUMMARY

1. During a study of the fungus parasites of the roots of peas and other legumes it has been found that the roots of nearly all our common leguminous crops, wherever grown, are extensively invaded by a characteristic fungus which has previously been known in a considerable number of plants as a mycorrhizal fungus.

2. The term mycorrhizal fungus as used here does not carry with it any implication that the association of the fungus with the higher plant is of benefit to the latter plant. In fact, there is some evidence which indicates that it is more or less injurious.

3. So abundant is this mycorrhizal fungus that it appears unlikely that many plants of alfalfa, clover, peas, and other legumes ever reach maturity without having their roots more or less invaded.

4. The fungus is found only in the primary cortex.

5. The mycelium is coarse, nonseptate in the roots, and though it passes through the outer cells when entering the root is chiefly intercellular in the deeper cells, advancing in long strands toward the growing end. It sends into the deeper cells haustoria which are often of complicated structure, filling more than half the lumen of the cell. The contents of many of the cells containing haustoria become greenish yellow, thus imparting a characteristic discoloration to the entire root. The taxonomic position of the fungus has not been determined, but it appears to belong among the Phycomycetes.

6. No culture medium has yet been found which appears to be capable of supporting the growth of this fungus independently of host tissue.

7. The new growth of perennial clover and alfalfa roots in early spring contains but little of this fungus, invasion occurring at least in part from the growth of the previous season. When root growth is retarded in midsummer, the rootlets become invaded almost to their tips. In autumn, when root growth is again rapid, the fungus is unable to keep pace with it and the new growth is largely free from invasion.

8. The fungus has been found able to invade roots of peas and sweet peas which are grown in a constant soil temperature as high as 33° and as low as 9° C. These temperatures approach closely the extreme limits at which these plants are able to grow.

9. In a single plat experiment clover, alfalfa, and sweet peas were found to grow more vigorously in soil in which this fungus had been killed by formaldehyde, but it is not certain that the absence of this parasite is the only factor which was responsible for the benefit.

10. A list of plants other than legumes in which this same type of mycorrhizal invasion has been found is given.

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OBSERVATIONS ON THE MECHANISM OF THE REACTION BETWEEN FORMALDEHYDE AND SERUM PROTEINS[1]

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That formaldehyde effects profound changes in the protein molecule is, of course, well known, for the use of formaldehyde as a tanning, fixing, and hardening agent depends upon its property of "denaturing" proteins. One phase of its denaturing action—that is, the effect of formaldehyde on the solubilities of the proteins of blood serum in ammonium sulphate solutions—was described in a previous paper (*4*)[2]. The present paper reports the results of a study concerning the mechanism of the changes which take place in the proteins of formolized serums.

In order to establish continuity with the preceding paper it seems desirable to give the following brief summary of the pertinent findings previously reported: (1) The addition of formaldehyde to blood serum caused a progressive decrease in the solubility of the proteins in ammonium sulphate solution. In that respect, the albumin behaved as if changed to pseudoglobulin; the pseudoglobulin as if changed to euglobulin; and the native and formed[3] euglobulins, in their turn, as if changed to proteins of still lower solubility. (2) With a given serum the rate of transformation was proportional to the concentration of formaldehyde, and with sufficient formaldehyde proceeded to the complete disappearance of the albumin and pseudoglobulin fractions. (3) The addition of sufficient formaldehyde to serum resulted in the formation of a gel. With given concentrations of formaldehyde the rapidity of gelatification was determined by (*a*) the concentration of the proteins and (*b*) the concentration of the salts.

The study of the mechanism of the reaction by which the more soluble serum proteins are converted by the action of formaldehyde into less soluble forms was directed along the following lines: (1) The effect of formaldehyde on the precipitation limits of pure globulins and albumins; (2) the order of the reaction which results in the conversion of the more soluble proteins into euglobulins; (3) the effect of temperature on the velocity of the reaction; (4) the effect of the addition of formaldehyde on the titrable acidity; and (5) the effect of formaldehyde on the hydrogen-ion concentration of the serum.

THE EFFECT OF FORMALDEHYDE ON THE PRECIPITATION LIMITS OF PURE GLOBULINS AND ALBUMINS

Schwarz (*15*) treated serum albumins and globulins with formaldehyde and found that the treated albumins and globulins were precipitated by salts which did not precipitate them before treatment. He did not undertake an exact study of the change which occurred, nor did he state the concentration of salts and proteins which he employed. In order to obtain more specific information the following experiment was made.

EXPERIMENT I

Euglobulins, pseudoglobulins, and albumins were separated as precipitates from a clear, normal, hog-blood serum

[1] Received for publication April 19, 1924—issued February, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 481–482.

[3] Native euglobulins = the protein of native serum which is insoluble in one-third saturated ammonium sulphate solution. Formed euglobulins = the proteins insoluble in one-third saturated ammonium sulphate solution formed by the action of formaldehyde on the more soluble proteins.

by appropriate treatment with ammonium sulphate. The precipitated fractions were dissolved separately and the salts removed by dialysis. After dialysis, NaCl in an amount sufficient to make a concentration of 0.9 per cent was added to each dialyzed fraction. As the volumes of these fractions were unequally increased by dialysis, identical concentrations of the separate proteins unfortunately were not obtained. The solutions thus obtained were examined on the day before the formaldehyde was added, and on the tenth, eighteenth, sixtieth, and ninety-fifth days thereafter. On the sixtieth day the amount of formaldehyde in the albumin fraction was increased to 0.37 per cent and on the ninetieth day to 0.74 per cent. The results of the examination are reported in Table I.

It is to be noted that the concentration of proteins in the albumin fraction was lower than in any of the other fractions. This test suggests that albumins, in the presence of formaldehyde, probably pass through the stage of solubility corresponding to pseudoglobulins before the stage corresponding to euglobulins [4] is reached.

This experiment has been repeated several times under practically the same conditions, and in each instance the results confirmed those presented above. The several experiments of this study may be summarized as follows: In the presence of 0.18 per cent formaldehyde pure native pseudoglobulins behaved as if they were converted into euglobulins, and pure native albumins as if converted into pseudoglobulins, but euglobulins were not produced from albumin solutions

TABLE I.—*The effect of formaldehyde on the precipitation limits of pure globulins and albumins*

Protein fraction	Time of contact	Grams per 100 c. c.		
		Euglobulins	Pseudoglobulins	Albumins
	Days			
Euglobulin	[a] 0	1.86	0.45	0.00
	10	Gelled.		
Pseudoglobulin	[a] 0	.08	2.78	.00
	10	.50	2.36	
	18	1.60	1.26	
	60	2.12	.74	
Albumin	[a] 0	.00	.15	1.73
	10	.00	.36	1.52
	[b] 60	.00	.80	1.08
	[b] 90	No examination was made.		
	95	.52	.98	.38

[a] Before the formaldehyde was added.
[b] The amount of formaldehyde was increased.

The euglobulin fraction before treatment contained a small amount of native pseudoglobulins and in this fraction gelatification occurred by the tenth day. In the pseudoglobulin fraction there was an increase in the formed euglobulins throughout the duration of the experiment. In the albumin fraction with 0.18 per cent of formaldehyde there was a progressive increase in the protein designated as pseudoglobulins, but even after 60 days there was no appearance of formed euglobulins; however, on increasing the formaldehyde in this fraction to 0.74 per cent on the ninetieth day there was a production of formed euglobulins. containing only 0.18 per cent formaldehyde. With greater amounts of formaldehyde, euglobulins were produced from albumins.

THE ORDER OF THE REACTION AS DETERMINED BY THE VELOCITY OF THE REACTION

In the paper previously mentioned, it was indicated that the velocity of the reaction involved in the formation of euglobulins in formolized serum was controlled by the concentration of the reacting substances, namely, the proteins of the serum on the one hand and the formaldehyde on the other. This

[4] Throughout this paper the unmodified term "euglobulins" is used to refer to any protein which is precipitable by one-third saturated ammonium sulphate whether it was originally present in the serum or was formed by the action of formaldehyde on serum proteins.

suggested the possibility of determining the order of the reaction. Accordingly, an experiment was performed for the purpose of measuring the extent of transformation of the more soluble proteins into formed euglobulins after they had been subjected to the action of formaldehyde for definite periods of time. From the resulting figures the velocity constant was calculated by the use of the monomolecular formula,

$$K=\frac{l}{t}\log_n\frac{A}{a-x}.^5$$

The values for K thus obtained were so irregular that it was concluded that the reaction was either not of the first order or that the simultaneous occurrence of side or consecutive reactions was obscuring the results. In order to avoid complexities of this character, recourse was had to Ostwald's (*9*) expedient of determining the order of a reaction. As employed in this study, this involved dilution of the original solution and the determination of the time required for the euglobulins in the diluted and undiluted solutions, respectively, to be increased by a definite percentage, or inversely, the time required for the noneuglobulin proteins of each to undergo a given amount of transformation into euglobulins.

As stated by Bigelow (*2, p. 361*), this method depends upon the fact that—

> if a reaction is of the first order, the time required to reach a given stage is not altered by altering the original concentration. * * * If it is of the second order the time required to reach a given stage is inversely proportional to the original concentration. * * * If it is of the third order, the time required to reach a given stage is inversely proportional to the square of the original concentration.

Thus, the time required for a given amount of work to be done in concentrated solutions on the one hand and half concentrated on the other may be expressed by the following ratios: For first-order reactions as 1 : 1; for second-order reactions as 1 : 2; and for third-order reactions as 1 : 4.

In formolized serum at least two proteins are converted into euglobulins, so at least two reactions must progress either simultaneously or consecutively. Therefore, since only one product, euglobulin, is formed and since the ratio between the time required for a given amount of work to be done in the concentrated and half-concentrated solutions is determined by comparing the rate of formation of this one product in the two solutions, the reactions involved in the transformation of the more soluble serum proteins into euglobulins are to be regarded as either side or consecutive reactions. Mellor (*7, p. 75*) states that—

> a reaction may be really compounded of two or more side reactions of the same order and yet have the same formal integrated equation as a normal una-bi- * * * molecular reaction.

Ostwald's method is designed to overcome the disturbing influence of side or consecutive reactions.

Provided that the reactions are side reactions and all belong to the same order, one of the above-named ratios should apply. On the other hand, if they belong to different orders and occur simultaneously, some intermediate ratio should apply, while if they are of different orders and occur consecutively, a change between the earlier and later ratios should take place. With these ideas in mind, the method of Ostwald was applied to the reaction which results in the formation of euglobulins in formolized serum.

EXPERIMENT II

To 190 c. c. of horse-blood serum of known protein content, and previously heated to 38° C., 10 c. c. of a formaldehyde solution, which contained 3.74 gm. of formaldehyde, were added. Simultaneously, to a second 190 c. c. of the same serum, 200 c. c. of a 0.9 per cent NaCl solution and 10 c. c. of the same formaldehyde solution (all previously heated to 38° C.) were added. Both portions were held at 38° C.; 10 c. c. samples of the first formolized serum and 20 c. c. of the second were withdrawn at frequent intervals, diluted to 200 c. c., primarily to stop the reaction, and the euglobulins determined at once. All determinations were made in duplicate. From the results obtained at each interval the amount of the more soluble proteins which had not been converted into euglobulins was calculated by subtracting the euglobulins found at the given time from the proteins originally present. The results are shown in Figure 1.

The time required for the more soluble proteins of the two serums to undergo a given degree of change was determined from Figure 1 by interpolation and is shown in Table II.

[5] For a full discussion of this method of determining the order of the reaction, known as the method of integration, any treatise of theoretical or physical chemistry may be consulted. In the equation for the first order reaction, K=a constant, known as the velocity constant; A=the original amount of the substance present; and X=amount of A transformed in time, t; $\log_n$=natural logarithm.

TABLE II.—*The ratio of the rate of the reaction in undiluted to the rate of reaction in diluted formolized serums held at a temperature of 38° C.*

Proportion of noneuglobulin protein converted	Minutes required to effect conversion		Ratio
	Undiluted serum	Diluted serum	
25	8½	16	1 : 1.9
33⅓	11	21	1 : 1.9
50	17	36	1 : 2.1
60	24	73	1 : 3.0
66⅔	33	107	1 : 3.2

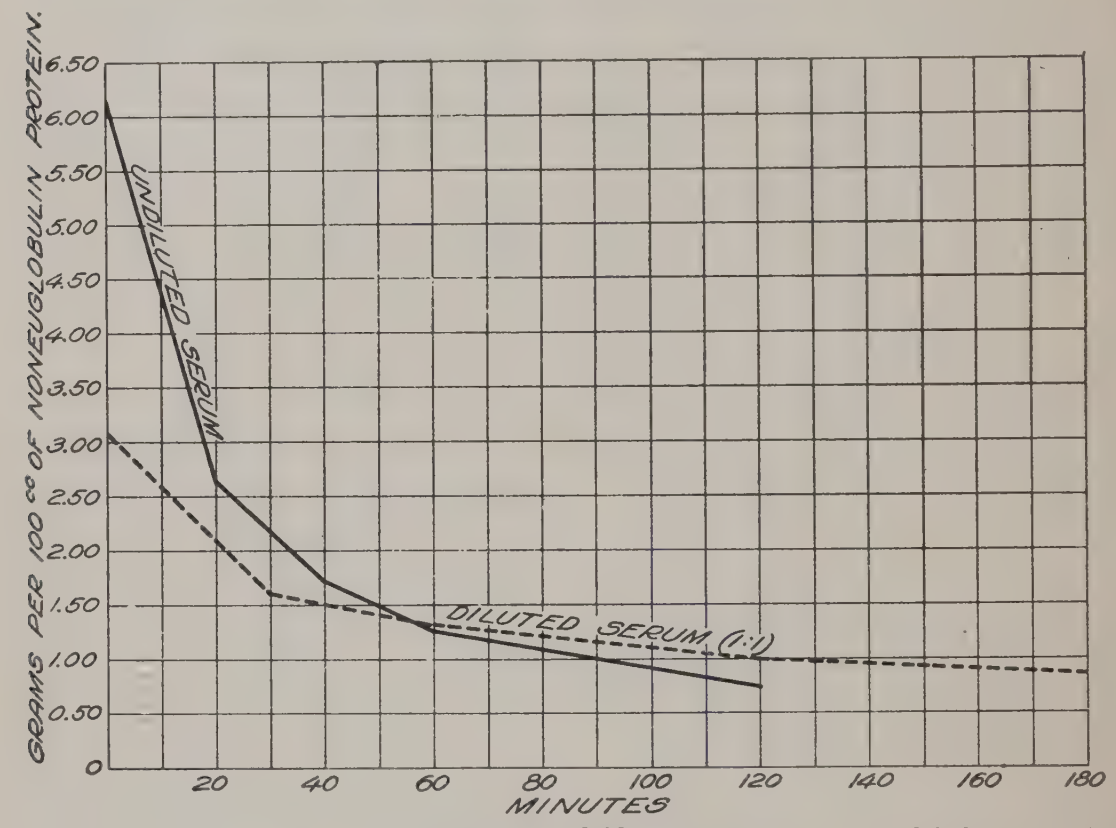

FIG. 1.—The rates of transformation of the more soluble serum proteins into euglobulins in undiluted and diluted formolized serums

By comparing the time required for a given amount of work to be done in the concentrated and in the half-concentrated serums (Table II), it will be seen that in the earlier stages of the reaction the ratio between the times required for a given amount of transformation to take place in the proteins of the concentrated and half-concentrated serums is approximately as 1 : 2. When the noneuglobulin proteins had been reduced by one-half an increase in this ratio takes place. Essentially the same results have been obtained in several other experiments. The probable cause of the change in this ratio will be discussed at the end of this section. It is regretted that pressure of other work did not permit an extension of this study to include solutions of pure pseudoglobulins and albumins; the results obtained, however, point rather strongly to the probability that the reactions involved in the transformation of the more soluble serum proteins of formolized serum consist, at least in the early stages, of reactions of the second order.

Confirmation of the probability of the reactions belonging to the second order was supplied unexpectedly by the following facts: Of the several formulas employed to determine whether a reaction is of the second order, one of them,

$$\frac{1}{t} \cdot \frac{x}{a-x} = ak,$$

applies only when the reacting substances are present in equimolecular proportions. In this formula t=time, a=amount of substance originally present, and x=amount of substance converted. In order to determine the values of t, a, and x a definite amount of formaldehyde was added to a serum of known composition. This was kept at a temperature of 25° C. Samples were taken at definite periods, the euglobulins determined, and from the values so obtained the velocity constant ak was calculated. The results are shown in the following Table:

TABLE III.—*The velocity constant of the reaction taking place in serum containing 0.74 per cent formaldehyde and kept at a temperature of 27° C*

[a]t	[b]x	[c]$a-x$	$ak=\frac{1}{t}\cdot\frac{x}{a-x}$
0	0.00	6.00	
15	.92	5.08	0.012
30	1.46	4.54	.010
45	1.87	4.13	.010
60	2.55	3.45	.012
120	3.30	2.70	.010
240	4.28	1.72	.010
360	5.00	1.00	.013
Average			.011

[a] t=time of contact.
[b] x=noneuglobulin protein coverted to euglobulins.
[c] a=original noneuglobulin protein.

The value for ak in Table III is a constant within the range of experimental error. Data from six other experiments in which undiluted serums were used, and in which t, x, and a had been determined were available. From these data ak was in each case calculated and the values so found were in each case constant within the range of experimental error. However, calculations of ak from the data obtained when diluted serums were used yielded decreasing instead of constant values. Thus, in the undiluted serum of Experiment II ak was found to have a value of 0.066 throughout the reaction, but in the diluted serum the values calculated for ak decreased from 0.033 to 0.015 in two hours. This suggests that the reaction in the diluted serum was approaching equilibrium, and this suggestion would account for the fact that a constant ratio was not obtained in Experiment II.

The fact that constant values were obtained for ak in the experiment reported in Table III and that a ratio of 1 : 2 was obtained in the early part of Experiment II points strongly to the probability that the reactions which take place in formolized serum belong to the second order even though the experimental data available may not absolutely establish such a conclusion.

THE EFFECT OF TEMPERATURE

The effect of temperature on the velocity of the reactions which result in the production of euglobulins in formolized serum was determined by the following experiment:

EXPERIMENT III

A horse-blood serum of known composition was divided into two portions, one of which was warmed to a temperature of 39° C. and the other to a temperature of 29°. When the serums had reached these respective temperatures a formaldehyde solution previously heated to the respective temperature was added to each in such amounts that each serum contained 1.85 per cent formaldyhyde. The serums were maintained at the respective temperatures for three hours, samples of each being taken at intervals for the determination of euglobulins. From the amounts of euglobulins found at each determination the amount of noneuglobulin protein remaining unchanged at each instant was calculated by subtracting the amount of euglobulins found from the total proteins present. The results are shown in Figure 2. From this figure the time required for given amounts of work to be done in the two serums was determined by interpolation and is shown in Table IV.

TABLE IV.—*The temperature coefficient of the reactions taking place in formolized serums as determined by comparing the times required for a given amount of work to be done in serums containing 1.85 per cent of formaldehyde and kept at 29° and 39° C., respectively*

Proportion of noneuglobulin protein converted	Minutes required to effect conversion		Temperature coefficient
	Serum at 39° C.	Serum at 29° C.	
25	7	18	2.60
33⅓	10	20	2.90
42	15	43	2.87
50	21	63	3.00
66⅔	41	140	3.41
70	65	180	2.80
Average			2.93

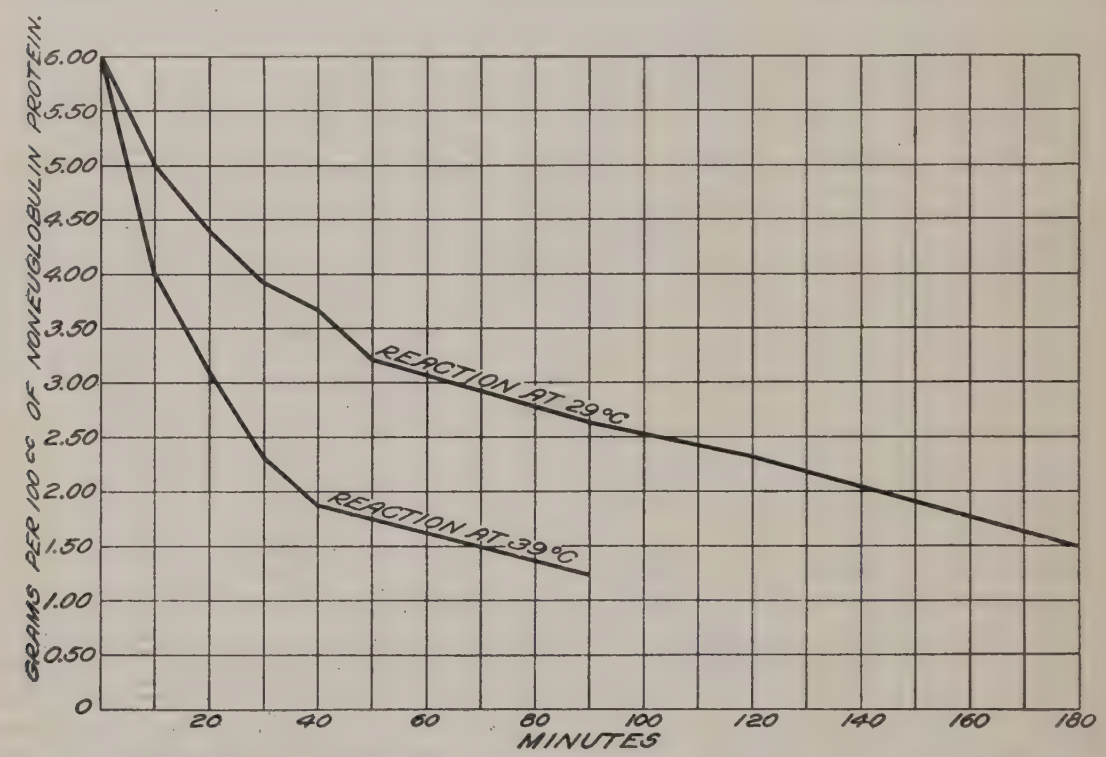

FIG. 2.—The rate of reaction in serums containing 1.85 per cent formaldehyde and kept at 29° and 39° C., respectively

An inspection of these results shows that the temperature coefficient of this reaction was approximately 3. This relatively high temperature coefficient eliminates the possibility of mere physical changes being responsible for the alteration in solubility brought about in serum proteins by the addition of formaldehyde, since the temperature coefficients of physical reactions are less than 2 (*13, p. 216*).

It is believed that the foregoing results show that the reaction in this experiment had a temperature coefficient of 3. However, in order to determine whether the same temperature coefficient could be obtained by comparing the velocity constants of the two reactions, the values for these were calculated by the use of the bimolecular formula in the manner previously described. The results are shown in Table V.

It will be noted in Table V that fairly constant values for *ak* were obtained for both serums, and that the value for *ak* obtained in the case of the serum kept at 39° C. is three times as great as for the serum kept at 29°. Thus by this method a temperature coefficient of 3 is also shown.[6]

[6] The serum upon which Table III is based and the serum shown in Table V as having been held at 29° C. both contained the same amount of protein and were held at about the same temperature. The two serums contained different amounts of formaldehyde; nevertheless, velocity constants, although not identical, were very similar. This suggests that the rate of reaction in formolized serums, provided an excess of the aldehyde is present, depends more upon the concentration of the proteins than upon that of the formaldehyde. There is also a suggestion that the formaldehyde itself does not enter the reaction of which the velocity constant was determined. These suggestions are in harmony with the observation that the addition of formaldehyde in excess may retard the rate of gelatification of serums.

TABLE V.—*The temperature coefficient of the reactions in formolized serums, determined by comparing the velocity constants of the reactions taking place in serums containing 1.85 per cent formaldehyde and kept at 39° and 29° C., respectively*

t	Serum kept at 39° C.			Serum kept at 29° C.		
	x	$a-x$	ak	x	$a-x$	ak
0	0.00	6.00		0.00	6.00	
10	1.98	4.02	0.049	.98	5.02	0.019
20	2.90	3.10	.047	1.60	4.40	.018
30	3.69	2.31	.053	2.07	3.93	.017
40	4.14	1.86	.055	2.33	3.67	.016
50	4.27	1.73	.049	2.77	3.23	.017
60	4.37	1.63	.044	2.93	3.07	.016
90	4.76	1.24	.043	3.37	2.63	.014
120				3.68	2.32	.012
180				4.53	1.47	.017
Average			.048			.016
Coefficient			3.0			1.0

THE TITRABLE ACIDITY OF FORMOLIZED SERUMS

Schiff (*14*) found that when formaldehyde was added to a solution of gelatin the titrable acidity increased as more formaldehyde was added. He also added unstated amounts of formaldehyde to albumin solutions and found that an immediate and relatively great increase in acidity was followed after 24 hours by slight decreases. Many observations upon the effect of formaldehyde on the titrable acidity of serums have been made by the writer, but as the results of all have been in accord, only one will be described.

EXPERIMENT IV

A clear, horse-blood serum was divided into four portions of 100 c. c. each. To these four portions there was added, respectively, ½ c. c., 1 c. c., 3 c. c., and 6 c. c. of a formaldehyde solution (37.34 per cent). Samples (10 c. c. each) were removed from each portion at the intervals shown in Table VI and titrated to a slight pink with N/10 NaOH, using phenolphthalein as an indicator.

These results show that following the addition of formaldehyde to a serum there was a rapid increase in titrable acidity. The speed and extent of this change were related to, but not proportional to, the amount of formaldehyde added. The primary increase in titrable acidity was followed by a gradual decline. The observations were discontinued at the ninety-sixth hour, so the full extent of the decline was not determined. Identical results have been obtained in a number of other experiments. However, in one serum which before treatment required about 20 c. c. of N/10 NaOH per 100 c. c. for neutralization, no increase in acidity was observed following the addition of formaldehyde, but only a constant decline.

TABLE VI.—*The titrable acidity of formolized serums*

Time of contact.	N/10 NaOH required to neutralize 100 c. c. of serum			
	With ½ c. c. formol	With 1 c. c. formol	With 3 c. c. formol	With 6 c. c. formol
	C. c.	*C. c.*	*C. c.*	*C. c.*
Before treatment	8	8	8	8
3 minutes	19	30	39	42
15 minutes	23	28	38	39
1 hour	21	26	33	37
3 hours	13	20	25	25
24 hours	9	11	20	20
48 hours	6	11	17	19
96 hours	5	10	15	18
124 hours				Gelled.

The most plausible explanation of the decline in acidity seems to be that this is due to neutralization of the originally free, and later liberated, COOH groups by basic groups which have not yet been attacked by formaldehyde. This explanation depends upon the possibility of basic groups existing in the presence of formaldehyde. That such a possibility exists has been shown by Schiff (*14*), who reported that relatively few NH_2 groups of proteins are combined with formaldehyde, and by Proctor (*10, p. 145*), who has shown that the reaction between amino-acid anhydrides and formaldehyde, and also between histidine and formaldehyde, is irregular; that is, not all of the NH_2 groups are combined.

If the assumption that the decline in acidity of formolized serums is due to a neutralization of the COOH groups of the protein by NH_2 or other basic N groups be accepted, then the reason for the applicability of the formula

$$ak=\frac{1}{t}\cdot\frac{x}{a-x},$$

which applies only when the reacting substances are present in equimolecular proportions, becomes apparent: proteins are composed of amino acids; and practically all amino acids contain basic and acid groups in equimolecular quantities.

THE P_H VALUE OF FORMOLIZED SERUMS

The results obtained in the titration studies suggest, of course, that the reactions which result in the transformation of the more soluble proteins of formolized serums into less soluble forms may be caused by changes in the hydrogen-ion concentration induced by the presence of added formaldehyde. The following two experiments bear on that question.

EXPERIMENT V

A hog-blood serum was divided into two portions. To one, 0.09 per cent of formaldehyde was added in the form of a solution of formaldehyde, and to the other 0.5 per cent of phenol was added. The euglobulins and the P_H value [7] of the serums were determined at the intervals shown in Table VII.

It will be noted that following the addition of formaldehyde and phenol, respectively, an initial decrease in the P_H value took place, and that this was in each sample followed by an increase. In the formaldehyde-treated fraction the euglobulins increased 124 per cent, while the phenol fraction increased but 24 per cent. This suggests that the changes in the solubilities of the proteins of formaldehyde-treated serums are not due solely to changes in the P_H value.

EXPERIMENT VI

A horse-blood serum was divided into two portions, to one of which 0.74 per cent and to the other 1.85 per cent of formaldehyde were added. The P_H value and the titrable acidity of these serums were determined at the intervals and with the results shown in Table VIII.

TABLE VII.—*The effect of formaldehyde and phenol on the P_H value and euglobulin content of hog-blood serum*

Added	Time of contact	P_H	Euglobulins (grams per 100 c. c.)
Nothing	Fresh	8.4	1.41
0.09 per cent formaldehyde	1 day	8.0	1.81
	1 month	8.3	3.16
0.05 per cent phenol	1 day	8.0	1.60
	1 month	8.5	1.75

[7] Determined colorimetrically by Dr. F. W. Tilley, of this division.

TABLE VIII.—*The changes in the titrable acidity and P_H values of formolized horse-blood serum*

Time of contact	Grams of formeldehyde added per 100 c. c. of serum			
	0.74		1.85	
	P_H	Acidity[a]	P_H	Acidity[a]
Before treatment	6.6	8.0	6.6	8.0
3 minutes	6.1		5.9	
1 hour	6.1	36.0	5.9	38.0
6 hours	6.1	28.0	5.8	30.0
24 hours	6.1	15.0	5.6	18.0
72 hours	6.0	15.0	5.6	15.0

[a] C. c. N/10 NaOH required to neutralize 100 c. c. of serum.

In this experiment the P_H value of the serum decreased and the titrable acidity increased on the addition of formaldehyde to the serum, and the changes in each were greater in extent when greater amounts of formaldehyde were added. However, while the immediate increase in titrable acidity was followed by a decline, no converse change occurred in the P_H value, which tended to decrease throughout the duration of the experiment. Attention is called to the fact that the serums reported in these two tables had different P_H values. The only explanation that can be offered is that the two serums were different, that they were derived from different species of animals, and that the serum with the lower P_H value had been kept longer before the experiment was begun. From the results of Experiments V and VI it is apparent that the addition of formaldehyde to a serum results in a decrease of the P_H value of the serum. It is also indicated that the change in P_H value of serums after formolization is not the sole factor controlling or inducing the transformation of the more soluble proteins into euglobulins.

MISCELLANEOUS OBSERVATIONS

In addition to the observations recorded in the preceding sections of this paper, a number of other observations regarding the behavior of the protein, including the factors influencing that behavior, have been made. It is not believed that the later observations have been substantiated sufficiently to warrant their presentation in detail. However, a few of these observations will be discussed briefly because they are considered of interest and importance.

Schwarz (*15*) found that when formaldehyde was added to serum a part of the aldehyde immediately, while another part gradually, combined. Similar results have attended experiments with formolized serums made in the hope of gaining information regarding, first, the relation between the rate of disappearance of uncombined formaldehyde and the rate of production of euglobulins, and, secondly, the relations between the amount of formaldehyde added and the amount combined. Thus, in several experiments 1.85 per cent of formaldehyde was added to serums. Following the addition of the aldehyde, the serums were examined at intervals for the purpose of determining the proportion of free formaldehyde which had disappeared from solution. In every test conducted in this way it was found that (*a*) a small amount, usually less than 0.33 per cent, disappeared, probably combined, within the first few minutes of the test, and (*b*) no increase in this amount occurred within the first five or six hours of the experiment. When the amount of aldehyde found to have disappeared in the first few minutes of the test was small, i. e., less than 0.20 per cent, and when the examination was repeated at the end of 24 hours, there was in some cases an indication that a slight additional amount of free formaldehyde had disappeared.

In regard to a relation between the amount of formaldehyde added to the amount combined: In a series of 12

experiments there was, with one exception, a greater amount of formaldehyde combined when a greater amount was added.

That the presence of salts is necessary for the formation of gels has been shown in the preceding paper. While the part played by salts has not been investigated, it has been noted that a greater part of the noneuglobulin proteins of the serum may be converted into euglobulins within a few hours and the serum yet fail to gel within many days. If salt is added to such formolized serums, they gel within a short time. This would suggest that the presence of salt, although necessary for gelatification, is not necessary for the transformation of the more soluble proteins into euglobulins.

DISCUSSION

It has been shown by Moll (*8*), Banzhaf (*1*), and others that an exposure of serum to a temperature of 50° to 60° C. for more or less prolonged intervals results in the transformation of albumins into pseudoglobulins and the latter into euglobulins. If the temperature to which the serum is exposed is sufficiently high, coagulation, of course, results. On the addition of formaldehyde to serum a similar transformation of the proteins occurs. If the concentration of the formaldehyde, other factors not preventing, is sufficiently high, gelatification results.

According to Chick and Martin (*3*), the reaction involved in the heat coagulation of protein increases in velocity with increase of temperature and they give 8 as the temperature coefficient of the heat coagulation of hemoglobin. The experiments reported in this paper indicate that the transformation by formaldehyde of the more soluble serum proteins into euglobulins increases in velocity with increase of temperature. The temperature coefficient is 3.

Mann (*6, p. 318*) states, and Sørensen and Jürgensen (*16*) confirm him, that any protein solution containing salts, acids, or bases becomes more alkaline on being coagulated. Although the addition of formaldehyde to serum results in a primary increase in acidity, this is, as has been shown herein, always followed by a decline in titrable acidity, and this decline may continue until the serum is more alkaline than it was in the beginning.

It is well known that native serums diluted with five or more parts of water do not coagulate on being heated, but if salt is added to such diluted and heated serums a coagulum is produced. This can be explained by the fact that heat coagulation of proteins consists, as shown by Chick and Martin and quoted by Robertson (*12, p. 307*) of two processes, (*a*) denaturation and (*b*) agglutination, and this occurs only in presence of salt. It has been shown in this study that the addition of formaldehyde to serum causes (*a*) denaturation, i. e., the conversion of the more soluble proteins into less soluble forms, and (*b*) gelatification, which latter condition, so far as the writer's observations go, occurs only in the presence of salts.

Chick and Martin (*3*) have shown that heat coagulation of proteins is not an instantaneous process, but that it proceeds with a definite velocity. In the work reported herein, it has been shown that the transformation by formaldehyde of the more soluble serum proteins into euglobulins, the end result of which is gelatification, is not an instantaneous process but that it also proceeds with a definite velocity.

From the foregoing it seems apparent that an analogy exists between the mechanism of heat coagulation on the one hand and of formal-gelatification on the other. However, at a certain point the analogy ceases, for while the reaction concerned in the heat coagulation of serum belongs to the first order, the reactions concerned in formol-gelatification seem to belong to the second order.

Robertson (*12, p. 309*) states that heat coagulation "is essentially a phenomenon of dehydration of which the first stage, that of internal neutralization through the loss of the elements of water from the end $-NH_2$ and COOH groups, probably corresponds to the phenomenon of denaturation while the subsequent or simultaneous polymerization of these anhydrides leads to the formation of particles so large as to assume the properties of matter in mass, i. e., flocculi." He states that Mann, Sutherland, Hofmeister, and Pauli have expressed similar views.

The points of similarity and contrast between the mechanism of the reactions which occur during heat coagulation and formol-gelatification have suggested a possible explanation of the latter phenomenon. It will be recalled that an immediate increase in titrable acidity followed the addition of formaldehyde to serum. This may be considered as the first stage in the reaction and may be explained, perhaps, by the occurrence of reactions resembling the reaction which takes place between amino acids and formalde-

hyde. It is, of course, well known that the addition of formaldehyde to amino acids results in a reaction which may be represented by the equation:

$$NH_2-R-COOH+CH_2O \rightleftarrows CH_2N-R-COOH+H_2O$$

Thus, the basic group of the amino acids is attacked by formaldehyde and so loses its pronounced basic character, while the acid character of the amino acid is correspondingly increased. It seems plausible to attribute the primary increase in acidity, which results from the addition of formaldehyde to serum, to reactions of that type; that is, in the first stage of the reaction in formolized serum, the formaldehyde may attack certain basic groups of the protein molecule as a result of which the protein molecule itself becomes acid in character. Such a reaction may be represented by the equation:

$$NH_2-R-COH=N-R-COOH+CH_2O \rightleftarrows CH_2N-R-COH=N-R-COOH+H_2O$$

Following the initial increase in titrable acidity, a gradual decline was found to occur. This may be considered as the second stage of the reaction. It is well known that NH_2- groups are not responsible for all the acid-combining capacity of the protein molecule, for all the NH_2- groups of the molecule may be removed by the action of nitrous acid and the molecule will still combine with acid. Because of this and similar facts, the reserve acid-combining capacity of the protein molecule has been ascribed by Robertson (*13, p. 156*) to the presence of enol, $-C(OH)=N-$, or similar linkages. To the nitrogen of this linkage is attributed the possession of two latent valencies, one positive and one negative. When the N is trivalent these two valencies neutralize each other; when pentavalent, they are capable of neutralizing a negative and positive radical, respectively.

$$-C(OH)=\overset{\overset{H}{|}}{\underset{|}{N}}-$$
$$\qquad\qquad\quad Cl$$

It is possible that the decrease in titrable acidity observed in formolized serums is due to reactions between such internal groups, on the one hand, and acid protein molecules, formed in the first stage of the reaction, on the other. The following formula represents such a possible combination:

$$CH_2-\overset{\overset{H}{|}}{\underset{|}{N}}-R-C(OH)=N-R-COOH$$
$$\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad OOC-R-N=(HO)C-R-N-CH_2$$

That a neutralization of the original and liberated $-COOH$ groups of formolized serums occurs seems assured. That the reactions described above offer a possible mechanism seems plausible. If this neutralization could be brought about by a combination of the acid groups with unattacked basic groups of the protein, then this neutralization could occur not only between adjacent basic and acid groups of the same molecule but between such groups of adjacent molecules. This would account for the finding that the reaction apparently belongs to the second order. We should thus have a polymerization which would be expected to continue until all of the protein molecules which had been attacked by the formaldehyde had united with other affected or unaffected molecules. Thus the proteins would acquire the properties of matter in mass and a network of the protein would extend throughout the serum, the visible result of which would be gelatification. That gels possess a network is accepted, according to Lloyd (*5*), by many as a fact. Reiner and Marton (*11*) have recently attributed the formol-gelatification of serums to polymerization, though without advancing confirmatory evidence.

This explanation of the mechanism of the changes which take place in the proteins of formolized serum is offered simply as a suggestion and with the full realization that the results presented herein are not sufficient to warrant at the present time a final conclusion.

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A BACTERIAL LEAFSPOT OF MARTYNIA[1]

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INTRODUCTION

Martynia louisiana is a coarse spreading annual with large pubescent leaves. The fruit is a capsule with a fleshy, hairy covering and long curved beak, which gives it the common name devil's claw or unicorn plant. It grows as a weed in the warmer parts of central United States and is also cultivated, the young fruits being used for pickles.

In July, 1922, diseased leaves of *Martynia louisiana* (*M. proboscidea*) were sent in by J. G. Lill, of the Office of Sugar Plant Investigations, from Garden City, Kans., (Pl. 1, A) where the plants were growing as weeds in and along the borders of sugar-beet fields. Bacteria were isolated from the leafspots but pure culture inoculations have shown that the organism isolated was not infectious to beets and was in no way connected with the spotting of the beet leaves. Finding no description in literature of a bacterial leafspot of Martynia, the following study of the disease as it occurred on the host plant and of the morphology, physiology, and cultural characters of the causal organism was made.

DESCRIPTION OF LESIONS

The leaves were heavily spotted with lesions mostly round but angular when bounded by veins. The infected tissue is water-soaked, sunken (Pl. 1, A) and translucent. The youngest spots are 1 to 2 mm. in diameter. The translucent centers are not more than 2 mm. in diameter; but narrow, swollen, brown margins, slightly raised above the surrounding tissue, develop as the spots become older. When several spots coalesce they form irregular patches of dry, light-brown tissue a few millimeters to a centimeter or more wide with translucent dots scattered over them. From sections (Pl. 2, A, B, and C) of these lesions bacteria stream in abundance.

ISOLATIONS AND INOCULATIONS

Isolations were made in two ways. Lesions were washed through 12 sterile water blanks, crushed in sterile broth and plates were poured. Other lesions were dipped in 95 per cent alcohol, then into $HgCl_2$ 1–1,000 for two minutes, washed through three sterile water blanks, crushed in sterile broth and plates poured.

Both methods gave practically pure cultures of a white organism which when sprayed on Martynia plants produced typical lesions of the disease (Pl. 1, B). Reisolations from these lesions again gave the same white colonies which again produced the leafspot when sprayed on Martynia.

Inoculations were made by spraying plants both in the field and greenhouse with water suspensions of the causal organism. Check plants were sprayed with sterile water. Inoculated greenhouse plants and checks were held in damp chambers three to four days. Four to six days after inoculation, lesions began to appear as water-soaked dots on the under surfaces of the leaves. Two or three days later the lesions may be a millimeter in diameter and visible on the upper as well as the under surface. The checks remained healthy.

In two greenhouse experiments infection appeared to progress through the veins, many of which, leading from marginal or other lesions, were water-soaked and darkened or more transparent than normal. Petioles collapsed at the point of junction with the blade and later entire leaves and petioles died and dried. From the petioles the infection passed into the stem until entire plants collapsed and dried up. In field experiments infection seemed to pass from growing tips through the stem into the more or less fully developed fruits, causing at first a water-soaked appearance. Later, this tissue turned brown, shriveled, and died

[1] Received for publication June 14, 1924—issued February, 1925.

Bacterial Leafspot of Martynia

PLATE I

(For explanatory legend see p. 485)

INOCULATIONS ON OTHER PLANTS

Injured and uninjured leaves of young beet plants were sprayed at two different times with water suspensions of the organism causing leafspot of Martynia. No lesions were produced.

Three attempts to produce infection on young cucumber plants in the greenhouse gave negative results. In one experiment Martynia leaves were sprayed at the same time as the cucumber plants and kept under the same conditions. Typical lesions developed in abundance on the Martynia leaves.

Leaves of the trumpet creeper, *Tecoma radicans* (L.) Juss., were also inoculated by rubbing with a water suspension of the organism. No infection resulted.

THE CAUSAL ORGANISM

MORPHOLOGY

The organism is a short rod with rounded ends, occurring singly or in pairs. Chains occur on 8-day beef peptone agar (Pl. 3, F). Measurements of organisms grown 24 hours on beef peptone agar and stained with gentian violet vary in length from 2.2μ to 1.3μ in length and from 0.7μ to 0.5μ in width, with averages of 1.68μ by. 59μ.

Capsules are present on beef peptone agar. (Plate 3, G). Sporangia and endospores are not formed. The organism is motile, having one to several bipolar flagella (Pl. 3, E).

The organism is Gram-negative.[2]

Smears from a 2-day beef peptone agar culture stained for 5 minutes in steaming carbol fuchsin and dipped for a few seconds in 20 per cent aqueous solution of H_2SO_4 were not acid-fast.

CULTURAL CHARACTERISTICS

Cultures were grown at room temperature unless otherwise noted.

BEEF PEPTONE AGAR SLANTS.—Growth is moderate, filiform, raised, glistening, somewhat contoured. There is a slight odor of decay, consistency is butyrous with traces of viscidity. The growth is white but may have a slightly opalescent tinge, and the agar turns slightly greenish brown. Numbers of small crystals form over and through the agar.

POTATO DEXTROSE AGAR SLANTS.—Growth is abundant, spreading, glistening, somewhat translucent, white by reflected light, but deep cream by transmitted light. Consistency is butyrous and there is no odor.

GELATIN STAB.—Held in the Altmann at about 20° C., growth is best at the top and extends only a few millimeters down along the line of puncture. Liquefaction is stratiform, beginning in 48 hours and completed in about 2 months. The liquefied gelatin becomes slightly fluorescent.

NUTRIENT BROTH.—A thin pellicle is formed with small crystals through it. When slightly jarred, the pellicle hangs down in long loops and strands from these crystals, which tend to stay on the surface. Clouding is moderate, being slightly heavier under the pellicle, and when cultures are undisturbed they often show definite bands of clouding. As cultures grow older the medium assumes a greenish tinge. Sediment is moderate in amount and granular.

NUTRIENT BROTH PLUS NaCl.—In beef extract broth with a P_H 6.9 clouding appeared in 2 per cent and 3 per cent NaCl in 48 hours; in 4 per cent after 4 days; and 5 per cent after 12 days. There was no clouding in 6 per cent or 7 per cent NaCl, but in both there was some bottom growth. Tests made in beef infusion broth with the same P_H gave the same results. Tests made in both infusion and extract broth with a P_H 8.2 gave clouding in 2 per cent and in one case in 3 per cent NaCl. A few chains occurred in 1 per cent NaCl and there were many long chains in 4 per cent and 6 per cent.

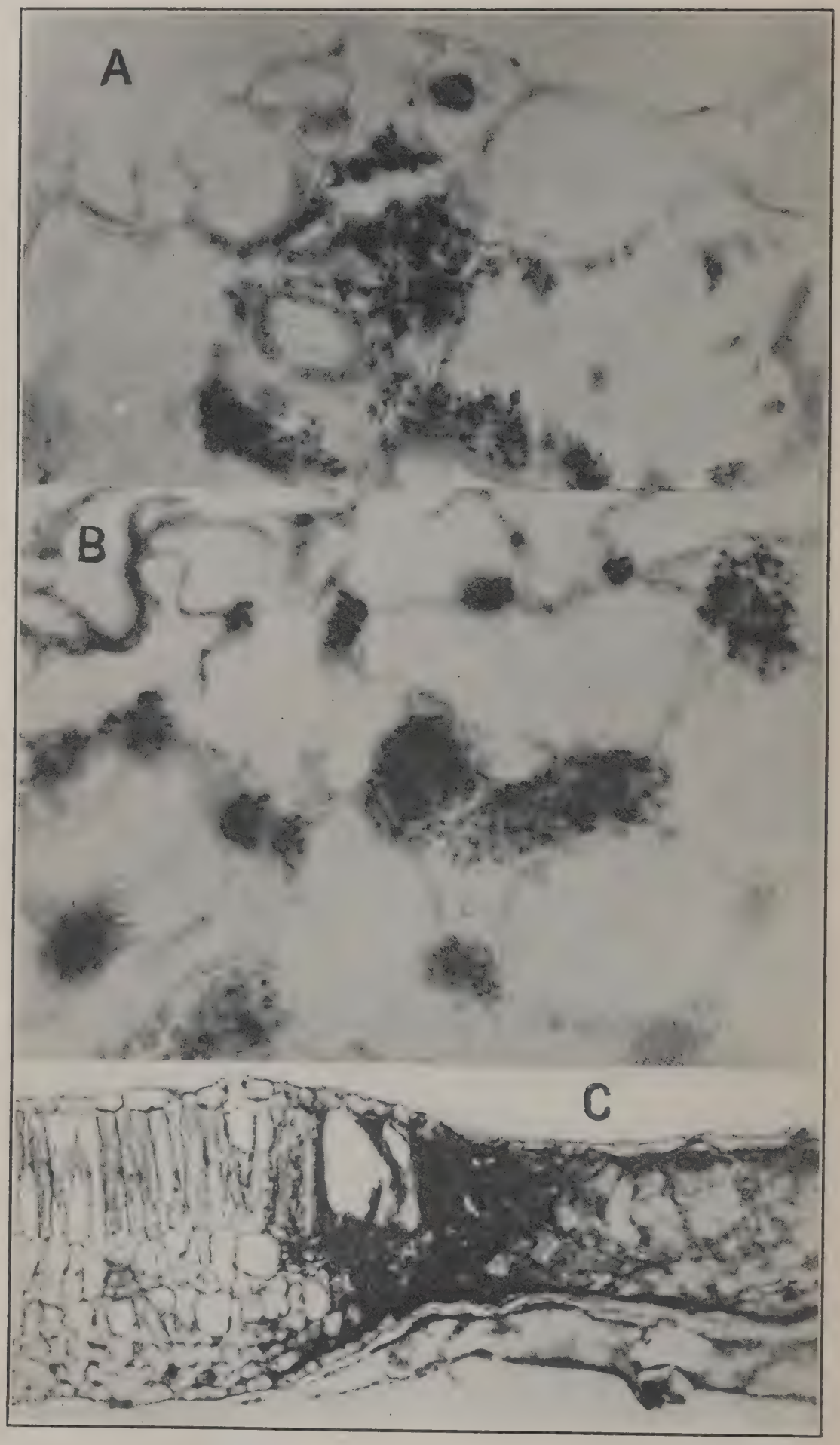
COHN'S SOLUTION.—Clouding is moderate and a thin pellicle forms from which hang large triangular crystals 2 to 4 mm. in length in 3 days. These crystals tend to pull down the pellicles, but crystals still hanging from the surface after 3 weeks are one-half to three-quarters of an inch in length. There was no green fluorescence.

[2] Methods I and II as recommended in "Manual of Methods for Pure Culture Study of Bacteria S. A. B.," were followed.

EXPLANATORY LEGEND FOR PLATE 1

A.—Natural infections on leaf of *Martynia louisiana* (= *M. proboscidea*) collected at Garden City, Kans., Aug. 31, 1922. Photographed by transmitted light Sept. 5, 1922. × 1. Photographic work by James F Brewer.

B.—Leaf of *Martynia louisiana* (= *M. proboscidea*) showing artificial infections. Sprayed Aug. 22, 1923, with water suspension of Culture I in field. Photographed by reflected light Sept. 7, 1923. × 1.



Bacterial Leafspot of Martynia

PLATE 2

(For explanatory legend see p. 487)

USCHINSKY'S SOLUTION.—Clouding is moderate, sometimes heavy with a ring or pellicle and after about a week the medium is light green. After about 8 weeks the cultures are a creamy yellow in color and have pellicles 2 to 4 mm. thick. These pellicles extend in viscid threads or swirls down into the liquid when shaken, but do not sink. There is a small amount of viscid precipitate.

FERMI'S SOLUTION. — Clouding is light. A heavy pellicle forms in a few days and the medium takes on a greenish tinge. After 2 or 3 weeks the pellicle may be 5 mm. deep. After about 8 weeks the cultures are a deep cream color and the pellicle hangs in long viscid strings or loops from the surface. There is no precipitate.

AGAR COLONIES.—Growth in 2 days is moderate, circular, smooth, glistening, raised, 1 to 2 mm. in diameter, with margins entire. By transmitted light fish scale markings become evident in young colonies but later disappear (Pl. 2, A). After 4 to 5 days colonies become umbonate (Pl. 3, C, D). In about a week they are 6 to 10 mm. in diameter in thin sown plates and the medium becomes greenish in tint. Older colonies become translucent.

GELATIN COLONIES.—Growth is moderate, circular, crateriform, the margin is somewhat filamentous, liquefaction is saucer-shaped, internal structure is granular filamentous. Liquefaction is evident in 1 to 2 days and is completed in about a week, depending upon the numbers of colonies on a plate.

BLOOD SERUM.—There is good growth in 48 hours, smooth, glistening, filiform, cream colored, butyrous. In 5 days the blood serum begins to clear under the cultures and becomes slightly depressed. After 2 to 3 weeks the serum is brown and transparent. The slant is thin and depressed under the growth.

PHYSIOLOGY

TEMPERATURE RELATIONS.—The optimum temperature for growth is about 25° C., the maximum about 37°, the minimum about 1.5°. The thermal death point is 49°.

OPTIMUM REACTION AND TOLERATION LIMITS.—The optimum reaction is between +15 and +20 or P_H 6.0 to 6.8. There was growth at +26, P_H 5.4, but no growth at +30, P_H 5.2. There was a trace of growth at −8, P_H 8.9, and no growth at −11, P_H 9.2.

TOLERATION OF ACIDS.—Isolation No. I grew in 0.1 per cent and 0.2 per cent of tartaric, malic, and citric acids, all cultures turning yellowish to bright green. The acids were added to neutral broth giving the following reactions:

Tartaric, 0.1, +10, P_H 7.2; 0.2, +23, P_H 6.0.
Malic, 0.1, +12, P_H 7.0; 0.2, +25, P_H 5.8.
Citric, 0.1, +13, P_H 7.0; 0.2, +26, P_H 5.9.

These acids were also added to uncorrected broth +20, P_H 6.1, to give definite P_H reactions, and cultures grew in the following reactions but not above them:

Tartaric, +29, P_H 5.2.
Malic, +28, P_H 5.4; +33, P_H 5.2.
Citric, +28, P_H 5.7; +33, P_H 5.4.

CHROMOGENESIS.—Nutrient broth, agar, and gelatin are slightly greened. Steamed potato cylinders become cream colored to tan.

INDOL PRODUCTION.—Cultures were grown in a solution of 1 per cent peptone, 0.5 per cent disodium phosphate, and 0.1 per cent magnesium sulphate for 17 days. Tested with H_2SO_4 and sodium nitrite, results were negative.

The same test was made in broth cultures grown for 4 days. Broth cultures 2 weeks old were tested by the Ehrlich and Salkowski methods recommended in the "Manual of Methods." There was no indication of indol production.

H_2S PRODUCTION.—Strips of lead acetate paper were suspended over broth, agar, potato and rutabaga cylinders. There was no discoloration of the paper.

Stabs were made [3] in media containing 5 c. c. of beer extract peptone agar and 5 c. c. of 0.1 per cent basic acetate. Growth at the surface gradually turned light brown, later medium

[3] Following the method recommended by the 1923 "Manual of Methods."

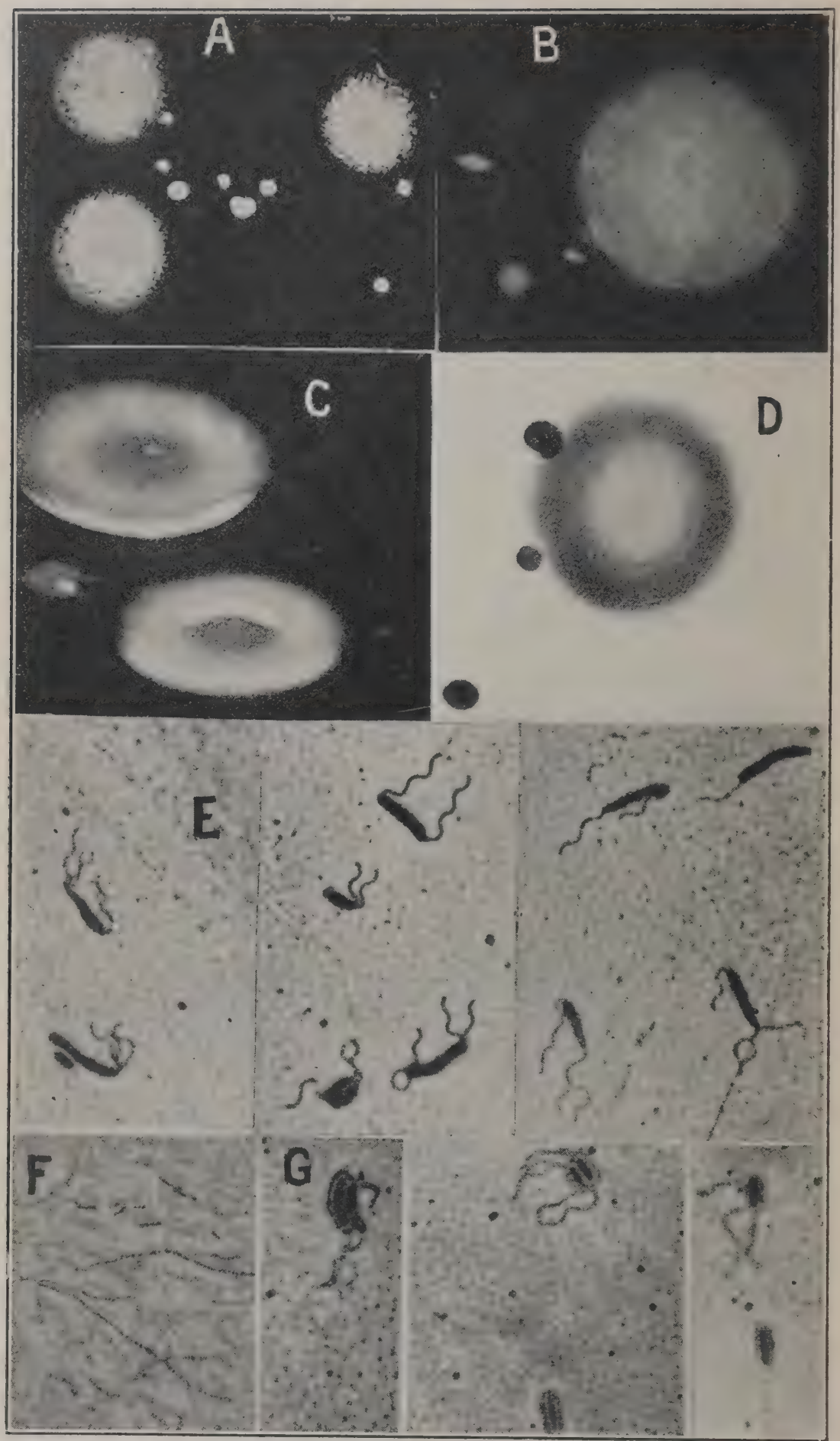
EXPLANATORY LEGEND FOR PLATE 2

Cross sections of infected leaves of *Martynia louisiana* (= *M. proboscidea*). Cut 5μ thick. Carbol fuchsin stain. Photographed Mar., 1924.

A.—Beginning infection. Substomatal cavity filled with bacteria. × 1900.

B.—Beginning infection with bacteria in intercellular spaces. × 1900

C.—Advancing margin of a 13-day old lesion. Infected tissue has collapsed. Bacteria are most abundant near the margin of the lesion and also appear outside the leaf tissue. × 180.



Bacterial Leafspot of Martynia

PLATE 3

(For explanatory legend see p. 489)

brown, then the agar became light brown in the upper half and finally after 2 months light brown throughout. A small amount of H_2S was produced.

RELATION TO OXYGEN.—Growth was observed in fermentation tubes containing 1 per cent peptone and 2 per cent sugars from which the oxygen had recently been driven off by heating. At the end of a week there was light clouding in the closed arms of tubes containing saccharose, dextrose, and mannit, but not in tubes containing maltose, lactose, and glycerin. In agar shake cultures containing 2 per cent sugars a few small colonies grew in the agar for the depth of half a centimeter in saccharose and dextrose only. The organism is aerobic.

DIASTASIC ACTION. — Streaks were made on beef peptone agar plates containing 0.2 per cent starch. Tested the second day with a saturated solution of iodine in 50 per cent alcohol there was no clear zone around any of the colonies.

Tested after 8 days there was still no cleared zone about the streaks. In some cases a zone 4 to 5 mm. wide was slightly more reddish than the surrounding agar. Twelve plates were tested and on one a fungus contamination had a definite cleared zone about it. There is very little diastasic action.

MILK.—There is evidently a delicate balance between the separation or nonseparation of whey and curd. When separation occurs the curd is evident after 2 days but is always soft. Litmus milk shows very slight acid reaction remaining about the same color as the checks with a slight reddish tinge. A test for rennet gave negative results. Cultures were heated for a half hour at 55° C. and then a few cubic centimeters poured into sterile milk. There was no separation of curd and whey when all the organisms had been killed. The curd is evidently an acid curd. Peptonization becomes evident in 2 to 3 days, progressing in definite bands, the clearest at the top. Peptonization is completed in about 2 weeks. Reduction of litmus begins in about 5 days and is complete in 8 to 14 days. When cultures containing reduced litmus are killed by boiling, the color returns a deeper shade than the checks but neither red nor blue. Reduction of methylene blue in milk is completed in 4 to 5 days. The blue color returns after the organism has been killed by heating the reduced culture. After several months milk turns amber to deep brown and becomes gelatinous or has gelatinous lumps suspended in the liquid.

NITRATE REDUCTION.—No gas is produced. Two tests for nitrites were made in nitrate broth cultures with sulphanilic acid and a-naphthylamine in 5 N acetic acid. Cultures showed good growth. Results were negative during the first few days but showed definite reduction after 2 to 3 weeks. Two tests for nitrites in the synthetic nitrate medium KNO_3 1 gm., K_2HPO_4 0.5 gm., $CaCl_2$ 0.5 gm., glucose 10 gm., distilled water 1,000 c. c. gave positive results. Reduction of nitrates is evident in this synthetic medium in 24 hours. There was no reduction in the checks.

FERMENTATION.—Tests were made in media containing 1 per cent peptone and 2 per cent carbohydrate or one-half per cent peptone and 1 per cent carbohydrate. Gas was not formed. Hydrogen-ion concentrations were determined colorimetrically and the results are given in Table I. Lactose showed no acid reaction, although tests were made after 1, 2, 3, 5, 6, 10, and 16 days, nor were acids detected from raffinose, maltose, mannit, rhamnose, or glycerin. Acid is produced from dextrose, sucrose, galactose, and arabinose.

LITMUS SUGAR AGARS.—Litmus showed reddening in dextrose, galactose, and arabinose after 24 hours; in sucrose in 3 days; in levulose a brownish red in 4 days. Mannit also showed a trace of this browning after 3 days, and when a month old all tubes were slightly redder than the checks. Lactose and maltose were bluer than the checks at the end of a week, and in 11 days the upper parts of cultures of arabinose were turning blue. In from 11 to 20 days partial reduction occurs in sucrose, maltose, levulose, galactose, arabinose, and glycerin.

EXPLANATORY LEGEND FOR PLATE 3

A.—Culture I, 2-day colonies on beef peptone agar. Photographed Mar. 9, 1923. × 10.

B.—Culture II, 3-day colony on beef peptone agar. Photographed Mar. 10, 1923. × 10. Showing the thin margins of young colonies. Transmitted light. Also buried and bottom colonies.

C.—Culture II, 8-day colonies on beef peptone agar. Photographed Mar. 15, 1923. × 10. Showing depressed centers. Reflected light.

D.—Culture II, 5-day colonies on beef peptone agar. Photographed Mar. 12, 1923. × 10. Transmitted light. Showing thin depressed centers and raised heavier borders.

E.—Culture I, 1-day beef peptone agar. Casares-Gil stain. Dec. 15, 1923. × 1900.

F.—Culture I, smear from 8-day beef peptone agar slant. Ribbert's capsule stain. Chains. Photographed Jan. 22, 1924. × 1400.

G.—Culture I, 2-day beef peptone agar cultures. Casares-Gil stain. Capsules. Photographed Jan. 24, 1924. × 1900.

TABLE I.—*Hydrogen-ion concentration of carbohydrate media inoculated with Culture I and compared with uninoculated controls*

Inoculation age (days)	Raffinose		Sucrose		Lactose		Maltose		Mannit		Dextrose		Galactose		Arabinose		Rhamnose		Glycerin	
	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check	Inoculation	Check
1	6.3	6.2	6.9	7.2	6.3	6.1	6.2	6.2	6.3	6.2	6.6	6.8	6.7	6.8	----	----	6.9	6.8	6.6	6.6
2	6.5	6.2	6.7	----	6.6	6.2	6.4	6.1	6.0	6.2	5.8	----	6.1	----	6.3	6.4	7.0	----	6.5	6.2
3	6.6	6.3	6.5	7.2	7.2	----	6.6	----	6.5	6.2	5.4	6.8	5.6	6.8	5.4	6.4	7.2	6.8	6.5	----
5	6.8	----	6.1	----	7.6	----	7.2	----	6.9	----	5.0	----	5.0	----	4.2	----	7.6	----	6.6	----
7	7.2	----	5.7	----	7.8	----	7.6	----	7.2	----	4.6	----	4.6	----	4.2	----	7.7	----	7.2	----
10	7.3	----	5.1	----	7.8	----	7.7	----	7.4	----	4.2	----	4.2	----	4.1	----	7.8	----	7.6	----
15	----	----	4.0	----	----	----	----	----	----	----	4.1	----	4.1	----	4.1	----	7.9	----	----	----
16	7.3	6.1	----	----	7.9	6.2	8.0	6.1	7.4	6.1	----	----	----	----	----	----	----	----	7.6	6.1

These results agree with those from the colorimetric tests except that in the colorimetric tests mannit and glycerin show no acid reaction. However, the colorimetric tests were not continued so long.

TECHNICAL DESCRIPTION

Bacterium Martyniae, n. sp.

A motile rod with rounded ends and polar flagella; single or in pairs, occasionally in chains; average measurements 1.68μ by 0.59μ; no spores; capsules occur; Gram-negative; not acid-fast; growth in nutrient broth moderate, turning light green with age, clouding in bands, with a thin pellicle containing many small rectangular crystals; clouding in Cohn's solution moderate with long triangular crystals growing to three-fourths of an inch in length; clouding moderate in Uschinsky's solution with a heavy pellicle; clouding light in Fermi's solution with a heavy pellicle; beef peptone agar colonies round, smooth, glistening, raised, later becoming umbonate, and turning medium green; gelatin liquefied; blood serum cleared and slightly liquefied; optimum temperature about 25° C.; maximum 37°, minimum 1.5°, thermal death point 49°; indol not produced; H_2S produced; aerobic; little if any diastasic action; soft acid curd formed in milk in 2 days; peptonization completed in 14 days; reduction of litmus beginning in 5 days and completed in 8 to 14 days; methylene blue reduced in 4 to 5 days; nitrates promptly reduced; acid without gas produced with sucrose, dextrose, galactose, arabinose; pathogenic on *Martynia louisiana* (= *M. proboscidea*), producing spots on leaves, and sometimes involving the entire plant.

CONTROL

Diseased leaves and plants should be destroyed as soon as lesions appear, and only seed from healthy pods should be used for planting when Martynia is grown for commercial purposes.

SUMMARY

Plants of *Martynia louisiana* (= *M. proboscidea*) showing bacterial lesions on the leaves were collected from beet fields in Kansas. Isolations from these lesions gave pure cultures of a white bacterium which readily reproduced the disease on healthy plants sprayed with the organism. When severe the disease may infect the fruits and even destroy the entire plant.

The name *Bacterium martyniae* is given to the causal organism. As a means of control, sanitary measures are recommended.

The investigation here presented indicates that *Bacterium martyniae* does not infect sugar beets, young cucumber plants, or the trumpet creeper.

RELATION OF SHEEP TO CLIMATE [1]

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INTRODUCTION

For many years sheep breeders have contended that breeding, feeding, and management are the chief factors involved in the further selection and development of the various breeds of sheep. But evidence from literature, together with the records of the university flock of the University of Illinois (at Urbana, Ill.), indicates that climatic conditions should also be considered.[3] The data here presented seem to support the following: (*a*) Sheep are limited to certain climatic conditions; (*b*) some breeds are better suited to certain climates than to others; (*c*) sheep are especially sensitive in lambing time and rutting seasons; (*d*) rutting season comes with falling temperature and varies from year to year; (*e*) high temperature with high humidity is detrimental to the growth of lambs; (*f*) some years are more favorable than others for lambing, growth of lambs, rutting season, and gestation period; (*g*) housing has extended the limits of sheep production; (*h*) some shelter types are more desirable than others. The climatic diagram methods used here were applied to tropical crops by Taylor (*25*).[4] The writer is not aware of a previous application of this method to a critical period by months and to good and bad years.

WILD SHEEP

The ancestry of domestic sheep is not definitely known. It is most probable that the Mouflon, or European wild sheep, and one of the races of Asiatic urial (*Ovis cycloceros*) have formed the chief parent stocks (Lydekker, *17, p. 27*). The Mouflon (*Ovis musimon*) is still found in Corsica and Sardinia. That at one time it had a wider distribution is evidenced by the remains of wild sheep in the superficial deposits of various parts of south Europe. The breeding or rutting season occurs in December and January. The gestation period is about 145 days, and the lambs (generally two) are born in April or May. The wild Mouflon interbreed with the domestic sheep and the offspring are fertile. The body covering of hair has to a minor degree the serrated scales which give to wool its felting property. The cry of the adult is a bleat very similar to that of domestic breeds (*17, 18, 19*). Sheep in the wild state are essentially mountain animals. They shun forests, live in the open, and feed upon the mountain grasses. The climatic conditions in such regions as the higher parts of Sardinia and Sicily are probably most suitable for sheep. The days are hot, the nights cool, the winters mild, with more or less rainfall; and the summers are dry (*2*). The sheep avoid the heat in the valleys by moving up the mountains, where it is cooler and where the grazing is less affected by the dry weather.

PRESENT DISTRIBUTION OF DOMESTIC SHEEP

Sheep are not uniformly distributed. There is dense sheep population in some localities and none in others (*6*). These areas of dense population have similar climates. The ideal climates here represented for sheep (fig. 1) are based upon these dense centers. The radical way in which such climates differ from others is emphasized by comparison with Taylor's 1919 diagrams. Each locality probably, though not necessarily, has the breeds which

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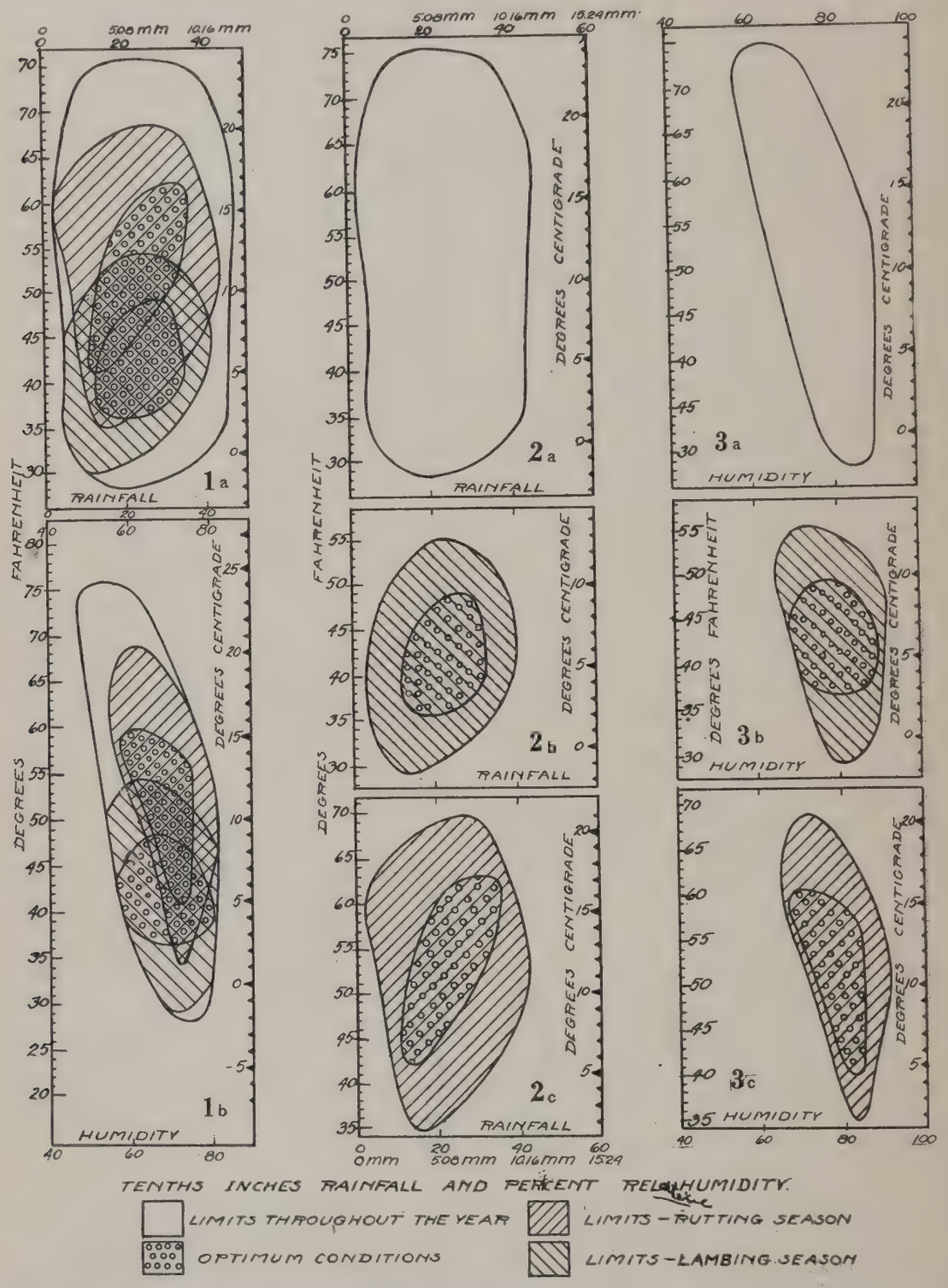
[2] The writer desires to express his appreciation to Dean W. C. Coffey, of the University of Minnesota, for suggesting the problem. He is indebted to Prof. H. P. Rusk and to W. G. Kammlade for many courtesies extended during the course of the investigation, especially in the securing of equipment and in giving access to the records of the university flock. The assistance from the graduate school research funds for the purchase of hygrothermographs to carry on a part of the work was invaluable. Thanks are especially due to Dr. V. E. Shelford for his encouragement and for suggesting and supervising the work with the graphic methods employed.

[3] A summary of the more important literature reviewed is contained in a manuscript in the library of the University of Illinois (master's thesis), where important sections are quoted from the original sources and full citations given.

[4] Reference is made by number (italic) to "Literature cited," p. 500.

thrive best, and best meet the needs of the people, as determined by time and varieties tried. Some breeds are suited to fertile valleys with damp climate and swampy soil; others are

In the new countries it was difficult to determine which sections were best suited to sheep and what breeds to select (*7*, *21*, *23*). Some were entirely unsuited; others proved suitable to



FIGS. 1 to 3.—1a and 1b, composite hythergraph and climograph of dense centers of sheep population in the world; 2b and 2c, composite hythergraph (temperature-rainfall) for critical periods; 3b and 3c, composite climograph (temperature-humidity) for critical periods

adapted to moorlands and mountains. Professor Wallace, of Edinburgh University, makes the following statement:

> When local conditions of climate, soil, management, and markets are suitable, the breed is likely to enjoy a fixity of tenure (*28*).

certain breeds only. At one time in the Eastern States the fine-wool sheep were crossed with the large, long-wool sheep, but the resulting cross was not very popular. The wool was too coarse, the carcass too large, and the large sheep, when fat, could not endure

hot summers. The many loose-wool sheep resulting from such crosses could not endure cold winter rains. The Down breeds were next used to replace the long-wool sheep, and the resulting crosses were more satisfactory (*27, p. 113*).

The sheep population has not yet reached large numbers in the Corn Belt, probably because of the limited pasturage, and because climatic conditions are unsuitable, as the summers are hot with frequently a rather high humidity. The Southern States have never had large numbers of sheep, and except in Kentucky and Tennessee the sheep are rather scattered. In general, they are small and angular, with a light fleece of poor quality. The fleeces of sheep introduced into these States deteriorate, and the mutton breeds lose their plumpness, evidently because of the need of covering and protection. R. Lydekker (*17, p. 24*) states—

> many tropical breeds of domesticated sheep retain a hairy coat comparable to that of wild sheep, but in other breeds, especially those inhabiting temperate and cold climates, this is more or less completely replaced by wool, except on the face, ears, and legs, where the hair is generally retained,

McKee (*19*) remarks that "sheep are not as good milkers in hot as in cold climates."

The sheepmen in the Western States maintain their flocks in the valleys during the winter and lambing season; then they move the flocks up the mountains, where they remain until fall. Occasionally heavy losses are incurred during these seasons because of the cold and deep snows. A flock of 100 sheep shipped to the Kadiak region of Alaska did fairly well during the summer on the luxuriant growth of grasses, although many suffered from foot rot. When closely confined for protection in winter, their fleeces dropped off in large patches, and emaciation followed (*3, p. 17*).

The sheep introduced into Colombia were the ordinary "Churro," or common Spanish breed. They flourish in the highlands but not in the hot valleys and plains. Unless the lambs are shorn early, the wool becomes loose, falls off in large patches, and is replaced by shining, close-lying hair similar to that of goats. This hair is never replaced by wool (*17, 247–250*). According to Griffith Taylor (*26*), "sheep [in Australia] occupy the warm inland drier belt and the cattle the wetter coastal regions."

On the north island in New Zealand, the Romney Marsh and the Lincoln breeds are found in large numbers, whereas the Merino is found only on the south island, where drier conditions prevail. The Romney Marsh breed comes from the county of Kent, in southeastern England, where the land is low and marshy. They are practically free from foot rot and can withstand cold wet weather. In New Zealand the conditions for the Romney Marsh are more favorable than in Kent, as evidenced by improved quality and length of the fleece. The Lincoln is quite similar to the Romney Marsh, although developed under somewhat different climatic conditions. In regard to moving sheep from one locality to another, Professor Wallace (*28*) states:

> There is an invariable rule that brooks no breaking that to thrive they must go from a poorer to a richer soil and more favorable climatic surroundings. For example, they must go from a humid into a drier district. It has been claimed that Oxford sheep coming from the cold, heavy clay of Oxfordshire and there subjected to the fogs and low temperatures do better in the drier and warmer localities. The long-wool and down breeds, if put on the mountain lands where the Cheviots, Blackfaces, and Welsh sheep thrive well, could not survive long enough to enable them to make the change. Lincolns, bred in Shropshire, do not attain the great size they do in their home country. The Kerry Hill (a Welsh breed), if removed to places where the soil is deep and the climate damp, get old and crocky or broken down in appearance and slack in their wool. It is claimed that the long wools thrive best on a light, dry soil, on account of the wool not getting so clogged in wet weather.

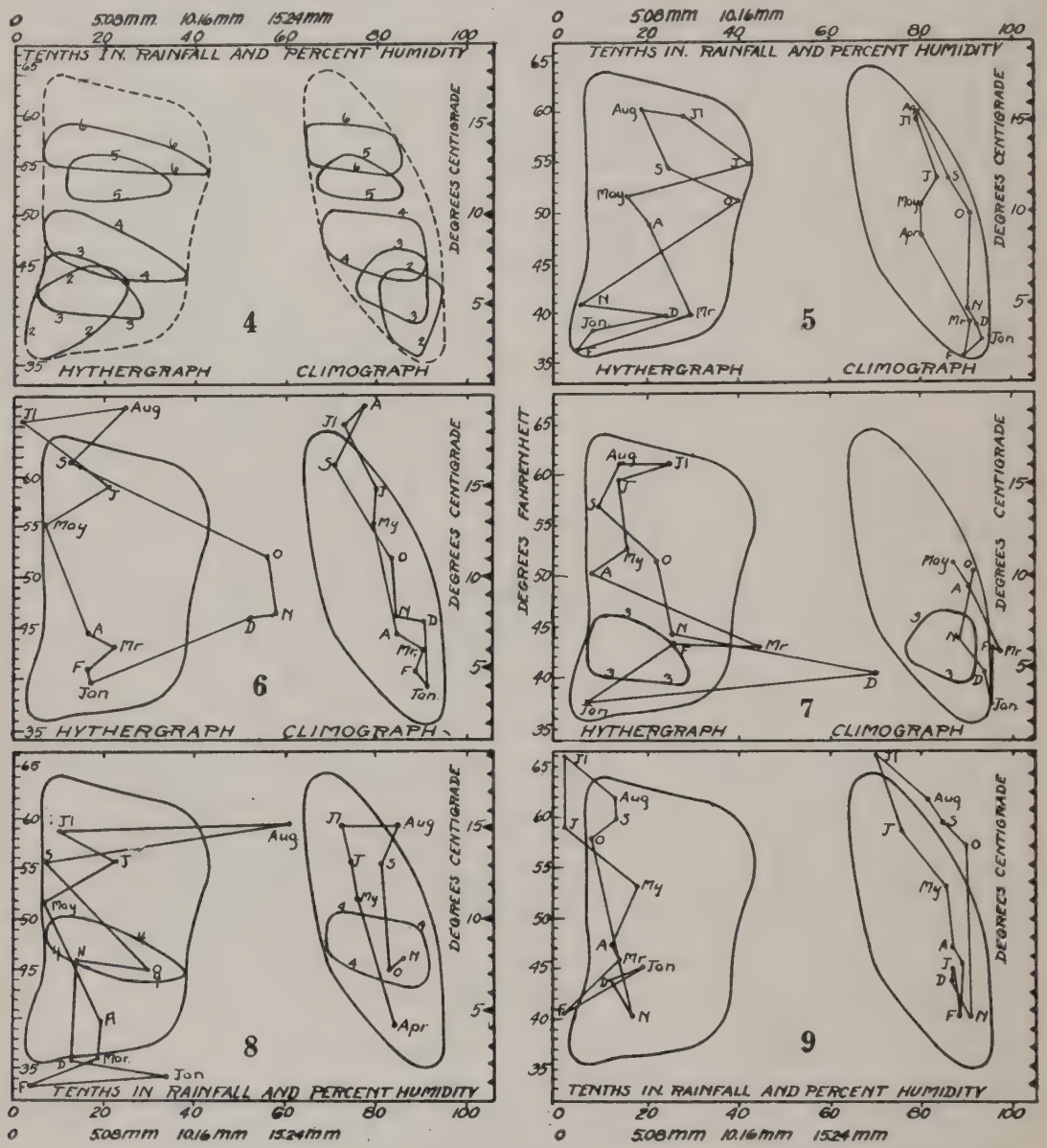
It has been assumed that sections having a dense sheep population have favorable climatic conditions for sheep production. Such dense world centers exist in South America (Uruguay and northeastern Argentina), South Africa (Basutoland), Australia (New South Wales), New Zealand, Great Britain, European Turkey, and Bulgaria. Russia and the United States also have a large sheep population (*6*).

COMPARISON OF CLIMATES

The climatic factors here considered are temperature, rainfall, and humidity. Comparisons were made by the use of the hythergraph (temperature-rainfall) (*13, 14, 15, 24, 25*) and the climograph (temperature-humidity) (*1, 22*). Graphs were made from the mean monthly temperature, rainfall, and humidity records for various meteorological stations located in sheep-growing sections. Each point on the hythergraph represents the mean of the temperature and the rainfall for a particular month. The points for each month in the year were plotted and then connected. For the climograph the mean monthly temperature and humidity were used. By this method

two factors and the months or critical periods can be shown at one time. In these graphs the temperatures are represented on the vertical scale to the left, and the tenths of inches of rainfall and the per cent of relative humidity on the horizontal scale at the top. (For a more detailed method of construction, see Taylor (*25*)). When 3a, and 1b). Graphs can thus be made of any section to compare with the composite.

A comparison of the graphs of the important sheep countries shows similarity in rainfall, temperature, and humidity (*2*, *5*, *10*, *11*, *12*, *20*, *29*). In general, the winters are mild and the summers cool, with a moderate amount



FIGS. 4 to 9.—4, limits for February, March, April, May, and June of a good sheep year in England; 5, good year, Reading, England, 1909; 6, bad year, Hastings, England, 1911, due to high temperature and humidity during July and August; 7, bad year, Reading, England, 1914, due to wet March and December; 8, bad year, Rounton, England, 1917, due to cold spring period of critical months; 9, bad year, Croyden, England, 1921, due to lack of rain, high temperature, and humidity in summer

possible, records from several stations with averages over as long a period of years as possible were selected for each section.

These graphs, representing the mean monthly temperature, rainfall, and humidity of the important (densest) sheep centers in the world, were made into a composite graph, made by tracing one graph on top of another and by connecting all the outer points (figs. 1a, 2a, of rainfall, sufficient to afford a good growth of forage; and the humidity is considerably lower in summer than in winter.

On the composite graph the rutting and lambing seasons have been plotted because sheep seem sensitive during these periods. The lambing season, which extends over three or four months, was obtained for each country and then plotted. The middle month (or months)

was taken as a mean because the bulk of the lambs are born at that time (figs. 1a, 1b, 2b, 3b). The rutting season was determined from the lambing season by counting back five months. The mean of the rutting season corresponds to the mean of the lambing season (figs. 1a, 1b, 2c, 3c).

The lambing and rutting seasons fall within comparatively narrow limits of temperature, rainfall, and humidity, and within narrower limits than does the composite graph for the whole year.

The graphs made from sections which have less dense population than those from which the composite was made show some variations—the climate may be hotter in summer, colder in winter; it may have more or less rainfall, a higher humidity, or a combination of these factors. The lambing or rutting seasons may not fall within the limits of those in the composite. For example, a hythergraph of Cedar Lake, King County, Wash. (fig. 14), for 1919 shows an excessive rainfall in the winter with 21½ inches in January, and a dry summer with 0.5 inch rainfall in July. There were no sheep recorded in this county, as the rainfall is too great in winter and too little in summer. Yet the temperature conditions are favorable, for the range is no greater than in some of the best sheep countries.

CRITICAL PERIOD

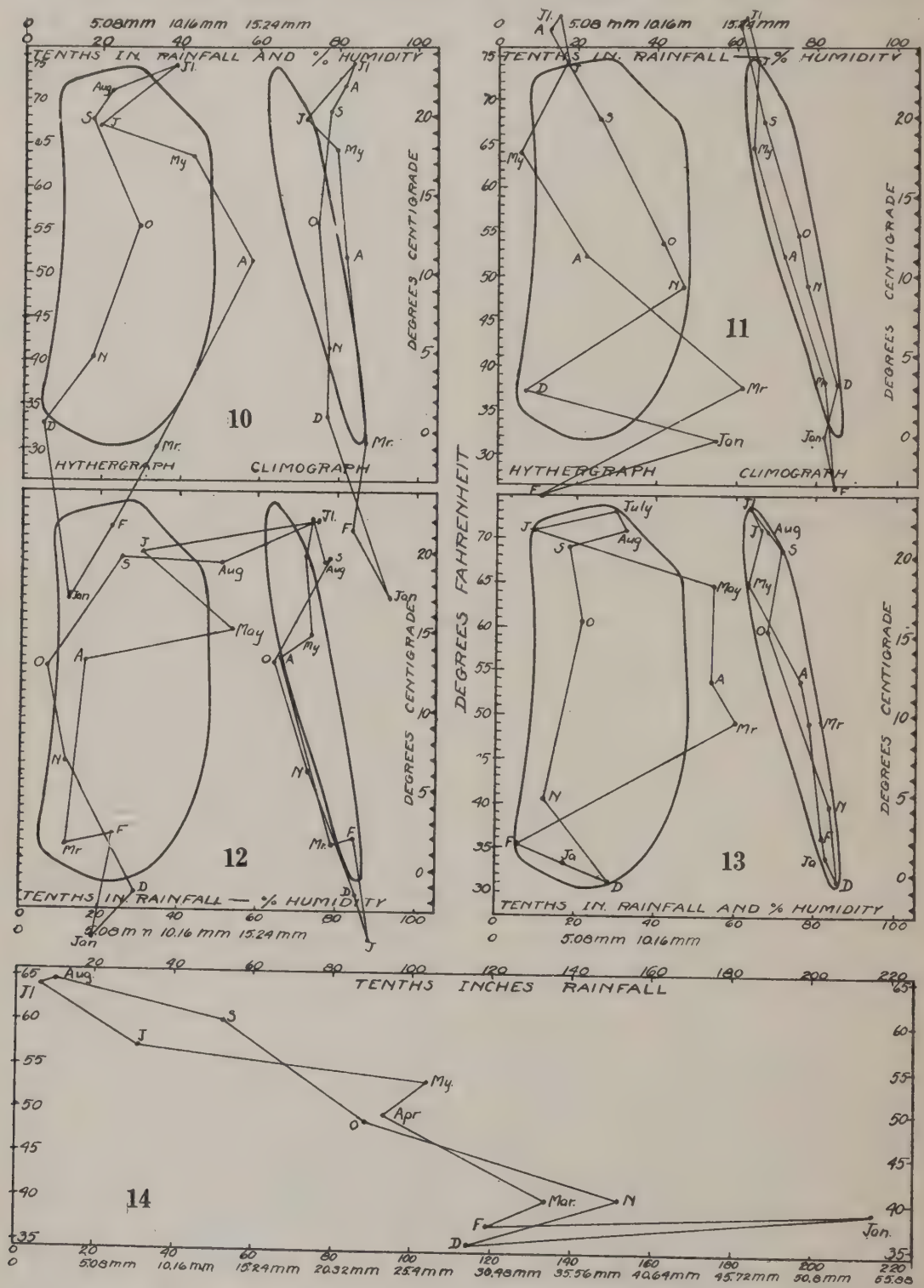
The periods in the life of a sheep during which it is more easily and more seriously affected by unfavorable conditions are the lambing and rutting seasons, which, as stated above, fall within narrower limits of temperature, rainfall, and humidity than the general conditions under which sheep live (figs. 1a, 1b, 2b, 2c, 3b, 3c). The first few months of the life of a lamb and to a less degree the time of pregnancy are also critical periods (fig. 4). A successful lambing season is often interfered with by cold, wet, or snowy weather, or by a shortage of feed. The new-born lamb is not able to withstand too rigorous climatic conditions. Likewise, the ewes, if exposed to excessive rainfall, or large amonts of snow, or if there is a shortage of feed, are liable to come to lambing time in unthrifty condition and give birth to weak lambs. According to Hammond (*8*), if conditions are extremely unfavorable, atrophy of the fetus, due to undernutrition, may occur. (This is much less frequent in sheep than in swine.)

The growth of the lamb during the first few months is readily retarded by hot, humid weather or by cold, wet weather. The extremely hot, humid weather also causes many lambs to lose weight (figs. 15 and 16). The late-born lambs are more severely affected than those born earlier. A breeder of purebred sheep in Illinois has found that clipping lambs in May has enabled them to withstand the summer conditions better.

According to Hammond (*8*), the rutting season comes with a falling temperature. It may be delayed by hot weather, particularly by warm nights (*4*). This becomes a serious problem in sections where the late summers and falls are hot, as it means that the ewes will not be bred until late in the fall, and the lambing season will be correspondingly late in the spring. Sections which have a warm fall usually have hot summers. In the West the ewes found unbred in the spring are gathered together and sent to the mountains, where a decreasing temperature is brought about by high elevation. Then they soon come in heat, and are bred for fall lambs. Conditions during the rutting season may have some effect upon the per cent of twins. According to Hammond (*8*), the number of ova produced by the ewes depends upon their condition at the rutting season. This is possibly the largest factor in the number of lambs born. (It is not true of swine, as many more ova are usually produced than can ever reach maturity.)

In Tennessee, where climatic conditions are generally favorable for lambing during the winter and early spring, the sheep men find it difficult to get their ewes bred early enough in the fall for early spring lambs. If they do not market the lambs before hot weather, the lambs cease to make profitable gains. In Illinois, unfavorable weather conditions are often experienced at lambing time, but can usually be overcome by proper methods of housing (*20*). Yet the lambs do not thrive quite so well during long spells of cold, rainy weather, even though adequately housed. When winters are extremely cold and the snow is deep, weak lambs and trouble at lambing time usually follow. The difficulty of getting the ewes bred early enough is quite frequent. The year 1915 was an exception, as the summer was unusually cool (fig. 12). Ewes came in heat during all the summer months, and the first lambs came in November. The summer months are generally very trying on sheep, and the growth of lambs, particularly those born late in the spring, is retarded. In England it

appears that unfavorable conditions are most likely to occur during the lambing season and in the winter months. The cold, wet weather is very trying to the ewes and lambs; and frequently a cold, wet fall and winter are followed by a small fall of lambs (*16*).



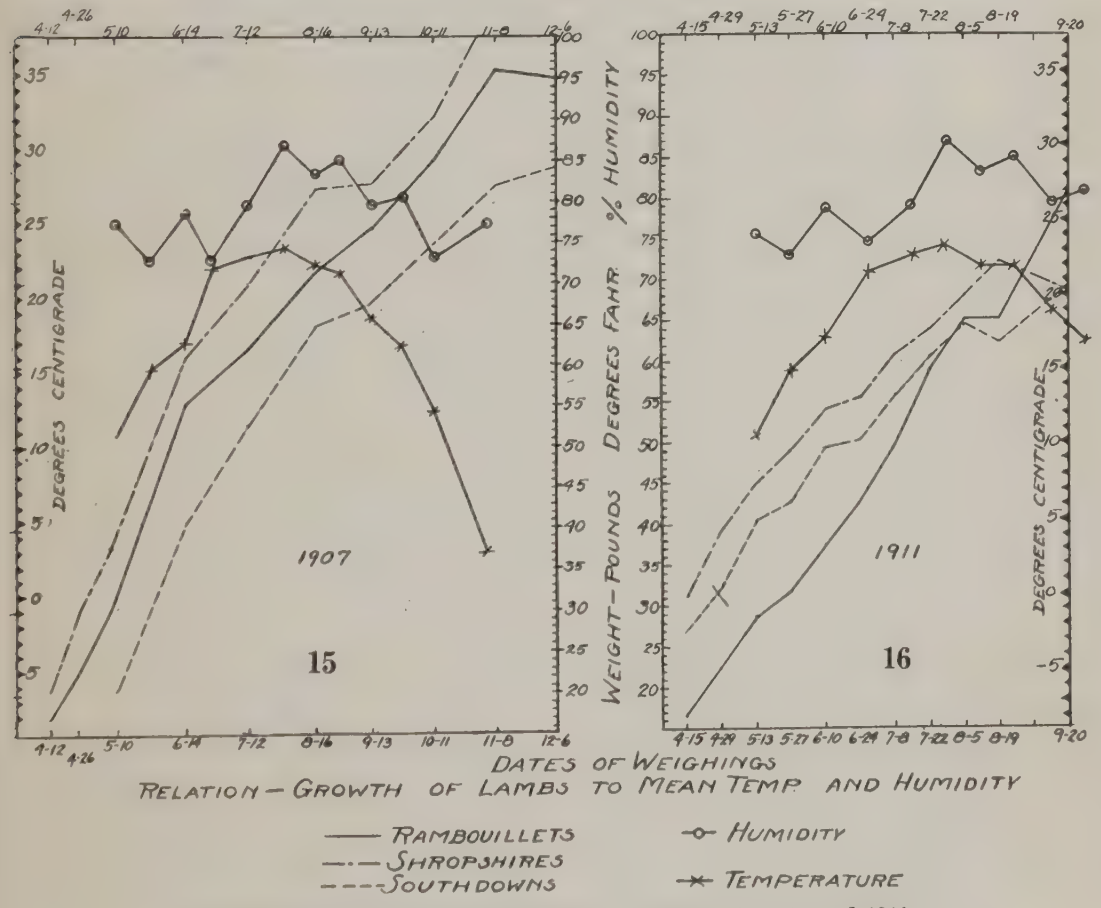
FIGS. 10 to 14.—10, bad year, Urbana, Ill., 1912, due to high summer humidities; 11, fair year, Urbana, Ill., 1913, due to low summer humidities; 12, bad year, Urbana, Ill., 1915, due to high humidity and rainfall; 13, good sheep year, Urbana, Ill., June, 1920; 14, hythergraph, 1919, Cedar Lake, King County, Wash.

GOOD AND BAD SHEEP YEARS

Since some years seem to be more favorable for sheep than others, a study was made of a number of years in south-central England and at Urbana, Ill. (*20*). Hammond (*8*) found the season to have considerable influence on the growth of sheep, particularly during their first year. He attributed most of this to rainfall, which he correlated with the weight at the end of the first nine months of the life of the lamb.

The years 1909 to 1921 (1912 no data) in England were studied, and the infor-

mation regarding the effect of each year upon the sheep was obtained (*16*). The years most favorable and unfavorable for the Southdown and Hampshire breeds were selected. Seasons unfavorable for these breeds were unfavorable for others in most districts in England. A number of meteorological stations were selected in south-central England, the native home of these breeds and the locality in which they are found in greatest numbers. Climographs and hythergraphs were made from the monthly means for the years 1909, 1910, 1911, 1914, 1915, 1917, 1918, 1919, and 1921. From the information at hand, the best lambing seasons and the most favorable summers, autumns, and winters were selected. From these were made a composite hythergraph and climograph, which represents a good sheep year in England. The unfavorable years can be compared with this good year and the contrast noted (figs. 6, 7, 8, 9). As the critical months of a good year fall within narrow limits, a graph was made for February, March, April, May, and June of the good years (fig. 4). For example, too cold an April of any given year, when compared with the composite hythergraph and climograph, might fall within the limits of a good year; but when compared with a composite containing the limits for April of a good year (fig. 4) it falls far below (fig. 8). A composite was made for the five months only, because they fall within narrower limits than some other months in the year, and come at more critical periods. The graphs for several years differ considerably, and do not always fall within the limits of a good sheep year for England. One critical month may be bad and others good. The graphs for 1909 at Reading, England, fall within the limits of the composite for a good year. That year was satisfactory for sheep (*16*), and in particular for the lambing season (fig. 5). The following selected case serves to illustrate bad years due to unfavorable critical months and to poor food and water supply. The summer of 1911 at Hastings was hot, and July was very dry; the humidity was high in July and August; and October, November, and December were very wet (fig. 6). This was noteworthy because of the small number of twin lambs, the failure of food crops, and the low price of mutton. From Figure 7 it appears that March, 1914, at Reading was too wet. Early lambs were weak and mortality was heavy. December of the same year was exceedingly wet, and ewes lost



FIGS. 15 and 16.—Growth of lambs at Urbana, Ill., 1907 and 1911

weight. April, 1917, at Rounton, England was too cold, as were January, February, and March. There was a difficult lambing season with a small lamb crop, but the later months were better. August was too wet, with over 6 inches of rainfall, but the humidity was not high. In 1921 at Croyden the lambing season was favorable (fig. 9). The summer was particularly dry, accompanied by humidity a little above the limits. Water had to be carted for livestock for six months.

A comparison was made between the good and bad years at Urbana, Ill., from 1906 to 1922 (*20*). (The information obtainable was not so complete as that for England.) The rate of gains made by the lambs was taken as an indication of the effect of the summer conditions upon them. The mean weights of each breed—Southdowns, Shropshires, and Rambouillets—were plotted for the years 1907, 1911, 1913, 1915. The weights were taken each month, and twice a month during some years. The mean temperatures and humidity for two-week periods were also plotted. Hammond has shown the normal growth of lambs to be regular. Figures 15 and 16 show a comparison of the growth or increase in weight with the mean temperature and humidity. The irregularities indicate that a high mean temperature accompanied by high humidity is correlated with the retarded growth of the lambs. In some cases there was a loss in weight for a two to four week period. The graphs presented here (together with others on file at the University of Illinois) show that retarded growth follows high temperature and humidity regardless of the date or the age of the lambs. Curves drawn for 1913 (on file at the University of Illinois) show comparatively uniform growth of lambs (fig. 11). The difficulty in making use of the weights of the university flock grew out of the different dates of lambing, evidently influenced to some extent by the weather condition of the preceding mating season. Mean growth was rendered insignificant by this irregularity, and retardation in growth was noted at critical periods regardless of the age of the lambs.

A mean relative humidity higher than 80 per cent and a mean temperature higher than 70° F. seem to be detrimental. The sheep can stand a rather high mean temperature if the mean humidity is not over 60 or 65 per cent. The rate of growth was more uniform in 1913 than for the other three years plotted. The study has not been carried far enough to give definite limits of temperature and humidity. The Rambouillets, which come from a country where the temperature runs higher than it does in the section from which the Shropshire and Southdowns come, seem to be less affected by unfavorable conditions. The late-born lambs are more seriously affected by unfavorable conditions than the early-born lambs.

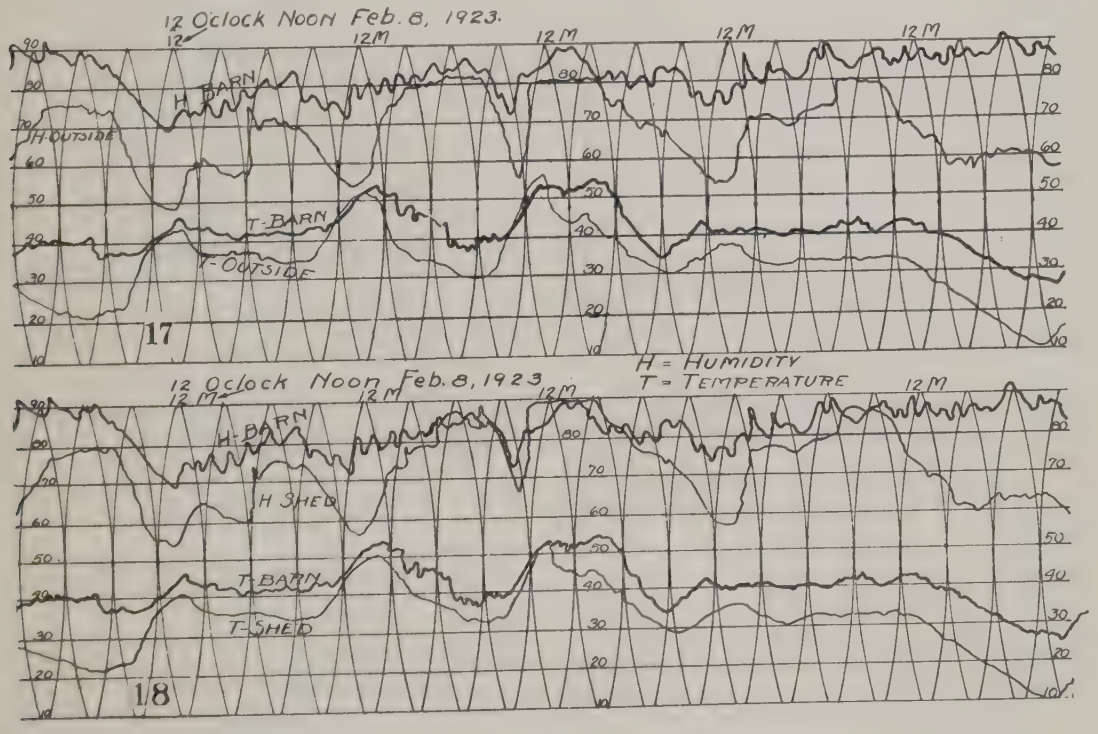
A good sheep year at Urbana, Ill., closely approximates the limits of a good sheep year in England, although it has a wider range, being warmer in summer and colder in winter. Figures 10, 11, 12, and 13 are hythergraphs and climographs of the years 1912, 1913, 1915, and from June, 1920, to June, 1921, respectively, at Urbana, compared with the limits of a good sheep year at Urbana. It was found that summers unfavorable for the growth of the lamb are rather common. The high humidity during the summer of 1912 was thus detrimental (fig. 10). During the hot summer of 1913, which was accompanied by a comparatively low humidity, the lambs made a regular and satisfactory growth (fig. 11). Although the summer of 1915 was cool and wet, the comparatively high humidity retarded the growth of the lambs, particularly of those born late in the spring (fig. 12). The period from June, 1920, to June, 1921, was a favorable year for sheep, and falls almost entirely within the limits of a good sheep year (fig. 13).

HOUSING OR SHELTER FOR SHEEP

The climatic limits under which sheep can be successfully kept may be extended by providing shelter and extra food. In the important sheep countries the sheep are seldom housed, and depend largely upon grazing for their food supply, although in some sections some additional food is provided during the winter. Shelter or housing makes it possible to raise sheep successfully in sections which have low temperatures, heavy snowfall, or excessive cold winter rains. The types of shelter may vary from sheds, constructed to break the wind and protect them from the cold rains, to warm well-constructed barns. In comparing graphs of these sections with the composite graph for good sheep countries, one should make some corrections for housing and various kinds of shelter. But no data have been found on how much correction is advisable.

The sheep farm at the University of Illinois has two types of building—one a shed which is open to the south,

the other an inclosed barn with a loft above for hay and feed storage. In order to make corrections for temperature and humidity, a hygrothermograph was placed in each building, and one out of doors, making possible a comparison between the shed, barn, and outdoor conditions. These hygrothermographs were placed in the pens where the sheep were kept; the one out of doors was in a shelter about 60 rods south of the buildings. All of the instruments were placed 12 to 15 inches above the ground because this distance is approximately the mean of the center of the body of the sheep when standing and lying down. The records from the three instruments began February 1, 1922, and have been continued to the present time.



FIGS. 17 and 18.—Comparison of temperature and humidity in barn, shed, and outside, Urbana, Ill., 1922

As yet there are not sufficient data to make accurate corrections for housing, but a marked difference is indicated by the records (fig. 17). The mean temperature was 3.4° to 10.2° F. higher in the barn than outside. The mean humidity was more variable, in some cases 13.6 per cent higher and others 6.7 per cent lower than outside. The differences between the shed and the outside were very small.

In the barn the temperature and humidity are more uniform with much less marked fluctuations than in the shed or out of doors (fig. 18). The barn is warmer than the shed in cold weather, but does not become warm so quickly on a mild sunny day in winter. The humidity runs higher in the barn, which frequently is steaming when opened in the morning. Some trouble from pneumonia and other respiratory diseases has been experienced with the sheep kept in the barn, but practically none with those kept in the shed. A study of the daily range and the maximum and minimum temperatures and humidities may also show a greater difference between the barn, shed, and outside. On many farms the housing is inadequate, with a resulting detrimental effect upon the ewes, especially where they are closely confined during very cold and snowy weather. After a winter of this kind, the lambs are usually born weak and as a consequence are less thrifty. This is possibly due also to a lack of exercise.

CONCLUSIONS

The dense centers of sheep population in the world are found within comparatively narrow limits of temperature, rainfall, and humidity. The mean temperature ranges between 28° and 77° F.; the rainfall between 0.3 and 4.5 inches per month; the relative humidity between 55 and 70 per cent at the higher temperatures and 65 and 91 per cent at the lower temperatures. Such conditions afford mild winters, cool summers, and sufficient rainfall to provide good grazing.

Climatic conditions must be favorable, especially during the critica periods of rutting season, pregnancy, lambing, and the growth of the lambs

The lambing period generally comes in the spring, enabling the lamb to get a good start before hot weather sets in. In the spring there is also an abundance of forage for the suckling ewe. The growth of the lamb is retarded by high temperature and humidity, by excessive rainfall and cold weather. The rutting season comes with a falling temperature and possibly a rather wide range between the maximum and minimum temperatures. Unfavorable conditions at the rutting season or during the gestation period tend to produce a small fall of lambs, and often weak lambs. If these critical periods are greatly disturbed, sheep are not likely to be numerous or profitable.

In Illinois, conditions of good sheep years are found to resemble the average conditions prevalent in the best sheep countries. The bad years generally have unfavorable conditions at some of the critical periods. The most serious condition here is the hot summer with a high humidity. No practical methods of overcoming these conditions are known. At Urbana, Ill., a mean temperature of over 70° F. accompanied by a mean relative humidity over 80 per cent, retards the growth of lambs. A higher temperature can be tolerated if the humidity is lower.

More attention should be given to finding breeds best suited to a locality, since the Rambouillets seem to stand the unfavorable conditions during the summer at Urbana, Ill., better than the Southdowns or Shropshires.

In some sections the limits of successful sheep production can be extended by proper methods of housing. The barn maintains a much more uniform and higher temperature and humidity than the shed or out of doors. But conditions in the barn are not entirely satisfactory for the health of sheep.

Sheep thrive best where cool summers and mild winters prevail and where sufficient rainfall is afforded to provide good grazing.

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TOLERANCE AND RESISTANCE TO THE SUGAR CANE MOSAIC[1]

By C. W. EDGERTON, *Plant Pathologist*, and W. G. TAGGART, *Assistant Director*, *Louisiana Agricultural Experiment Station*

A survey of the literature of mosaic diseases discloses a rather general belief among pathologists that (1) a plant once affected with mosaic is always so affected and (2) that with favorable environmental conditions an affected plant shows a gradual deterioration. There are a few recorded exceptions, but the small number of these makes only more prominent the general belief.

Where plants are propagated by seed, a condition of this nature is not particularly important, since apparently but few mosaic diseases are transmitted from one year to the next through seed. But where plants are propagated vegetatively, if this belief is true, the resulting conclusion is serious, for with plants like sugar cane or potatoes which are propagated by cuttings or tubers, a plant once affected must always remain affected, and after a sufficient length of time should show a deterioration that would make it unfit for planting. The only hope in such a situation would seem to be to grow healthy plants in some section where the mosaic did not spread rapidly and thus to keep a supply of healthy plants for propagation purposes. Such a course might be possible with potatoes but it would be impossible with sugar cane in a State like Louisiana, where the requirement for planting is from 3 to 5 tons per acre. If with such a crop the spread of the mosaic is extremely rapid—as it is on sugar cane in Louisiana—a 100 per cent infection must soon be reached, and with no healthy cane for planting we should expect from the general belief that there would be a gradual deterioration and possibly the failure of the crop unless fortunately a resistant variety could be found which might be substituted for the susceptible ones now being grown.

If, on the other hand, we consider conditions as they actually are, we find that in countries like Java, where sugar cane has been infected with mosaic for many years, a decidedly different situation. Instead of the canes being in bad condition we find what are called "tolerant" canes, canes that are affected with the mosaic but are not injured to a great extent. In many of the tropical sugar countries where the mosaic disease has been severe in recent years the Java seedlings are replacing the old standard varieties because of their tolerance to the mosaic. We also occasionally find in other countries the occurrence within a variety of a strain which is markedly resistant or immune. Possibly the Toledo cane which was reported from the Philippines in 1923 is an example of this.[2]

From the experiments and observations on the mosaic disease in Louisiana made during the past several years there have developed a number of things which have a bearing on the general mosaic problem and which also may be important in the control of mosaic diseases on vegetatively propagated plants. These results have a direct bearing on the general statement made in the introductory paragraph of this paper.

Soon after the recognition of the mosaic disease on sugar cane in Louisiana it became evident that conditions were excellent for its rapid spread and that it was useless to try to stamp it out or even to keep it under control. The Louisiana workers realized that the disease would spread rapidly across the State and would soon reach a point of maximum infection. As a matter of fact, the spread has been faster than was even anticipated at that time. Furthermore, experiments to test the possibility of keeping the disease in check by roguing the fields proved futile. Confronted with the idea that a plant once affected is always affected and that plants affected with a mosaic disease show a gradual deterioration, a solution of the problem did not seem remarkably hopeful. However, several lines of investigation were started.

[1] Received for publication May 24, 1924—issued February, 1925.

[2] HIND, R. R. TOLEDO CANE, A MOSAIC-IMMUNE VARIETY. Sugar Cent. and Plant. News. 4:105–107, 110, illus. 1923.

RAPIDITY OF NATURAL INFECTION OF CANE MOSAIC

The first work attempted with the sugar cane mosaic was to test out the different varieties of cane that are grown in Louisiana. It was very soon determined that the common commercial varieties, including D 74, D 95, Purple and Striped, were very susceptible to the mosaic, and that if healthy cane is planted in a badly infected field the crop will show practically 100 per cent infection before the end of the first season. It was also determined that diseased canes of these varieties when planted produced diseased plants the next year. This is in accord with the prevailing opinion in regard to mosaic transmission. In Table I are given the results of a few tests showing the rapid increase of mosaic on some of the susceptible canes when grown in a badly infected field.

TABLE I.—*Rate of increase of mosaic infection on susceptible canes*

Year	Variety	Percentage of mosaic on different dates				
		May	June	July	August	September
1920 [a]	D 74				100.0	
1920 [a]	D 95				100.0	
1921	Palfrey	4.4		56.6	100.0	
1922	do	1.9	36.6	47.0		(b)
1922	Purple	0.0	42.1	63.0		(b)
1922	D 74	1.8	17.8	64.0		(b)

[a] Most of the data for 1920 was lost by fire.
[b] The cane was examined in September, 1922, but since all of the plats showed practically 100 per cent infection counts were not made.

RESISTANCE TO INFECTION

It was noted, however, that cane of another variety known as L 511, acted in a manner different from the common canes. The L 511 is one of the canes developed at the Louisiana Experiment Station. It is very rich in sucrose, but, since the tonnage is usually light, it is not becoming generally planted. Plants of this variety when affected with mosaic show the disease in a form as severe as that of any of the other varieties, but only a moderate percentage of the plants in a field show the presence of the disease. One of the reasons for this has been determined to be the resistance of the plants to infection. Resistance to infection should be kept distinct from the relative severity of the disease after infection occurs.

The L 511 is one of the canes which shows the mosaic on the stalks as well as on the leaves. Healthy stalks are a light green in color but diseased ones have red stripes running in the direction of the main axis. For a number of years stalks lacking these stripes, that is, stalks free of the mosaic, have been selected and planted and the crop watched through the following year. The increase in mosaic on these plats is shown in Table II. In all cases the increase in mosaic has been very slow as compared to that on susceptible cane varieties. This shows a resistance to the infection but has no reference to the severity of the disease following infection. The stalks that became affected showed the disease in as intense a form as do susceptible varieties. To what this resistance to infection is due has not been definitely determined. It may, however, be the result of a better protection of the growing bud. The cluster of leaves at the top of a stalk of L 511 cane is much more compact than that on most varieties, and it is possible that this hinders the insects which carry the mosaic from attacking the young bud.

TABLE II.—*Rate of increase of mosaic infection on L 511 cane*

Year	Percentage of mosaic on different dates					
	May	June	July	August	September	October [a]
1921	2.2		21.5	55.0		43.7
1922	13.6	8.9	19.0		24.0	22.6
1923		9.2	10.3		12.3	9.2

[a] The October counts were made at the time when the cane was being cut for planting and were based on the number of stalks that showed the presence of red stripes. The other counts were based on the quantity of leaf infection apparent on the growing cane.

SELECTING FOR MOSAIC TOLERANCE

The work on the problem of sugar-cane mosaic which is considered by the writers to be the most important is the attempt to obtain more resistant or tolerant strains of our common commercial varieties. It is recognized that it is difficult to change or to improve a plant that is propagated vegetatively, yet there seeemed to be two possibilities worthy of testing. (1) Will sugar-cane varieties which show a moderate susceptibility or a moderate tolerance to mosaic vary enough, possibly by bud variation, to produce some plants or strains which will become more tolerant than the rest? Previous work on vegetatively propagated plants, such as potatoes, has shown that but little can be done in improving a variety, and it seemed very doubtful if any selection would improve the resistance of sugar cane. (2) Will a plant that is affected with mosaic, if it is propagated for a number of years, gradually acquire more resistance or possibly an immunity to the disease? The acquiring of immunity is a common thing in human and animal diseases, but no example of a plant acting in a similar manner has ever been demonstrated. It would seem, however, if a plant could acquire an immunity to any disease it would be to a mosaic.

Experiments to test these two possibilities were begun in the fall of 1920. At planting time selections were made in fields of D 74 and Purple cane in a region that had had for several years a 100 per cent infection of mosaic. Stools showing the least injury from mosaic were selected. No cane showing a total freedom from the disease was used, for healthy cane was not desired. What was wanted was infected cane showing a minimum amount of damage. This cane was planted by the side of unselected cane in a field showing a maximum infection. In the fall of 1921 the best cane from the selected plat was used for planting, and from this in the fall of 1922 the cane showing the least damage from mosaic was again planted. The results for the three years 1921, 1922, and 1923 are now available. Counts of the mosaic were made at frequent intervals and the comparative yields were obtained for the last two years. The results are shown in tabular form.

RESULTS WITH D 74 CANE

On the basal or younger portion of a D 74 leaf the mosaic shows in the rather usual form of somewhat lighter colored stripes or areas, but as the leaves grow out portions of these stripes tend to fade out and appear in the form of light-colored, sometimes nearly white, dots or slightly elongated spots (Pl. 1, A, *a*, *b*). On canes showing least damage from mosaic these spots may be so reduced during the latter part of the growing season that it is difficult to say whether the mosaic symptoms are present (Pl. 1, B, *a*). With this variety canes bearing the smallest and fewest light-colored dots or areas were selected for planting. The results obtained from the selection work with the D 74 variety are given in Table III.

The contrast between the selected and the unselected rows in the field was much more striking than appears in the figures in the table. The selected rows were darker green in color and the canes were not only larger but had stooled more. An examination of the individual plants showed that those in the selected rows did not have the mosaic symptoms in nearly so marked a form as in the unselected. Although all of the plants were known to be affected at the time some of the examinations were made, it was absolutely impossible to be certain that mosaic symptoms were present on some of them. While it is difficult to show sugar-cane mosaic in a black-and-white illustration, an attempt has been made in Plate 1, A and B, to bring out the contrast between the leaves from the selected and the unselected rows. These illustrations were made from photographs taken on September 15, 1922. Plate 1, A, shows the white dotted appearance very common with this variety and also the smaller dots on the leaf from the selected row. In Plate 1, B, which illustrates the more common condition of the leaves in these tests, the white dots from the selected row (*a*) are very few while the mosaic symptoms on the leaves from the unselected rows (*b* and *c*) are very obvious. These leaves do not show an exaggerated comparison, but are somewhere near the average.

How much of the 32 and 34 per cent increases in yield shown in 1922 and 1923 is due to the reduced effect of the mosaic is a question. It may be claimed that other factors of health and vigor were unconsciously considered when the selections were made each year. For the 1923 test no stool was used unless it contained at least four good stalks. There is little doubt, however, that a portion of the increased yield was due to the less serious damage caused by the mosaic.

TABLE III.—*Results obtained from selecting D 74 cane for tolerance to mosaic*

Year	Selection	Percentage of mosaic during the season				Yield in tons per acre
		May [a]	June	July	September	
1921 [b]	Unselected	40.0		63.0		
	Selected	30.0		28.0		
1922	Unselected	88.7	100.0	100.0	100.0	10.58
	Selected	58.0	94.1	90.0+	100.0	14.00
1923	Unselected		96.8	100.0	100.0	8.85
	Selected		85.9	100.0	(c)	11.90

[a] The percentages for May are too low on account of the very many small plants on which the mosaic could not be estimated satisfactorily.

[b] For 1921 the figures show only the percentages of pronounced mosaic. Most of the data for that year were lost by fire.

[c] A satisfactory count could not be made in September, 1923, since so many of the selected plants showed the disease in so mild a form.

TABLE IV.—*Results obtained from selecting Purple cane for tolerance to mosaic*

Year	Selection	Percentage of mosaic found during the season				Yield in tons per acre
		May [a]	June	July	September	
1921 [b]	Unselected	45.0		95.0		
	Selected	25.0		52.0		
1922	Unselected	93.6	100.0	100.0	100.0	17.37
	Selected	69.1	75.9	90.0+	(c)	19.89
1923	Unselected		100.0	100.0	100.0	18.5
	Selected		82.2	100.0	(d)	19.7

[a] The percentages for May are too low on account of the very many small plants on which the quantity of mosaic could not be estimated satisfactorily.

[b] For 1921 the figures show only the percentages of pronounced mosaic. Most of the data for that year were lost by fire.

[c] A satisfactory count could not be made in September, 1922, in the selected plat since less than 10 per cent of the plants showed clear-cut mosaic symptoms.

[d] A satisfactory count was impossible in September, 1923, in the selected plat on account of the poorly developed mosaic symptoms.

RESULTS WITH PURPLE CANE

On the Purple cane the mosaic appears in the ordinary light-colored stripes which are characteristic of this disease on many varieties. On severely affected plants these stripes constitute more than 50 per cent of the leaf surface and, consequently, the plants have a yellowish appearance rather than the normal dark green. Selections were made in the same manner as with the D 74 variety. The results are given in Table IV.

The contrast between the selected and the unselected row in the Purple plats was more striking than in the D 74 plats. The selected rows were dark green in color and had the appearance from a little distance of being perfectly healthy. Since these plants were also taller, there was a striking difference in the appearance of the different rows. At some seasons it was impossible to make mosaic counts on the selected rows because of the poorly developed mosaic symptoms. On a great many of the plants even at the

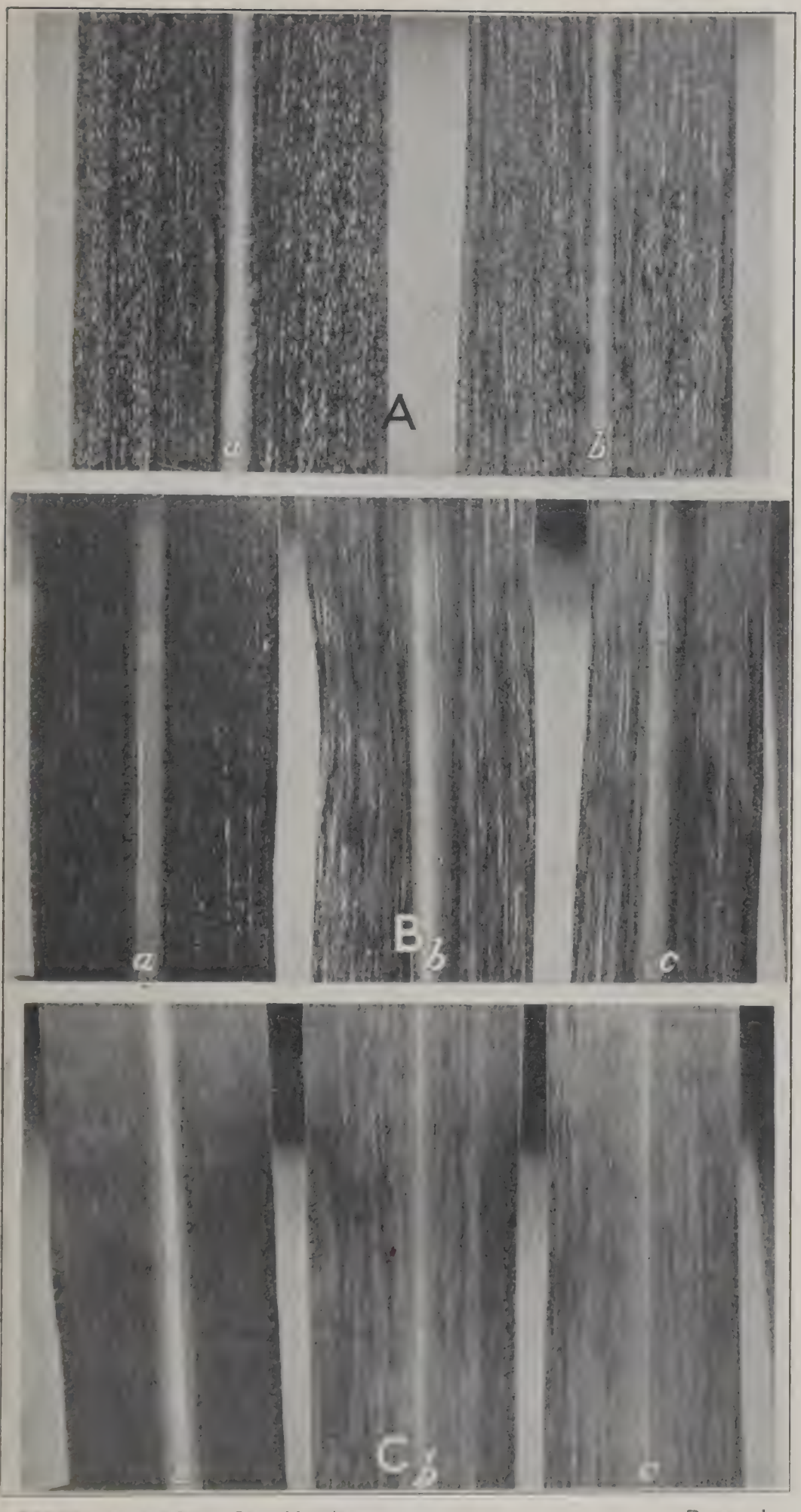
EXPLANATORY LEGEND FOR PLATE 1

Mosaic appearance of sugar cane leaves from plats planted with selected and unselected cane:

A.—The white dot effect of the disease on D 74 cane; *a* from the selected cane, *b* from the unselected.

B.—Average condition of D 74 leaves from selected and unselected plats; *a* from the selected cane, *b* and *c* from the unselected.

C.—Average condition of the leaves of the Purple variety from selected and unselected plats; *a* from the selected cane, *b* and *c* from the unselected.



Resistance to the Sugar Cane Mosaic

PLATE I

(For explanatory legend see p. 504)

season when the symptoms were most pronounced the mosaic could only be found by examining the basal or younger portion of the leaves. As the leaves grew out the light-colored stripes disappeared and the whole leaf surface took on a normal dark green color.

In Plate 1, C, are shown leaves from the selected and the unselected rows on September 15, 1922. All the leaves are from mosaic-affected plants, but none of the symptoms are apparent on the leaf taken from the selected row. The selected rows also showed a somewhat larger yield and it is probable that this increase was largely due to the decreased damage from mosaic.

DISCUSSION

The tests with the D 74 and the Purple canes have demonstrated that in fields with a 100 per cent infection plants can be selected that show a marked tolerance to the mosaic. How this has come about is as yet not clear. It is uncertain whether this is due to slight variations of the host with the variants more resistant, or whether the plant is gradually acquiring an immunity somewhat similar to acquired immunity in man and animals. As far as usefulness in the control of the mosaic is concerned, it is immaterial which explanation is correct.

If resistant strains are developing in nature, it is only a matter of time until the low-yielding, highly susceptible plants will be eliminated. Each year the percentage of stalks from tolerant canes would be greater and naturally a greater percentage of such would be used for seed. There is some evidence that this may be taking place in some sections of Louisiana where the oldest infections occur.

By discarding the susceptible and selecting only the most tolerant canes for seed, it is possible that our present varieties can be developed to a tolerant condition in a much shorter space of time than if natural selection is allowed to take its course.

This paper is not an argument for allowing the disease to take its natural course in all sugar countries. In those regions where the disease can be kept under control by roguing or by the use of clean seed it would be folly to let the mosaic go and try to select resistant varieties, but in places like Louisiana where no clean seed is available and roguing has been a failure this offers a possible solution of the problem, at least until satisfactory resistant varieties become available.

FURTHER STUDIES ON THE RELATION OF ONION SCALE PIGMENTATION TO DISEASE RESISTANCE [1]

By J. C. WALKER, *Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture,* and CARL C. LINDEGREN, *formerly Research Assistant in Plant Pathology, University of Wisconsin*

STATEMENT OF THE PROBLEM

In earlier studies of the nature of parasitism and resistance associated with the smudge disease (*Colletotrichum circinans* [Berk.] Vogl.) of onion bulbs (*3*, *4*)[2] it has been shown that the host tissue contains certain substances which are toxic to the invading organism. These substances fall into two classes, (a) the volatile oils and certain associated nonvolatile compounds in the cell sap (*6*) and (b) the toxins associated with scale pigmentation. The volatile substances which arise from the succulent tissue, especially upon wounding, are readily taken up by liquid suspensions of conidia and when present in sufficient concentration completely inhibit germination and retard growth. No marked consistent difference in the amount of these volatile toxins given off by different varieties of onion has been noted, and the conspicuous resistance of colored-bulb varieties as compared with the white types is not to be explained on this basis. The volatile substances, while generally toxic to fungi, apparently are not so effective upon rapid bulb-decaying organisms, such as *Botrytis allii* and *Fusarium cepae*, as upon milder parasites, such as *Colletotrichum circinans*. It has been suggested therefore that these toxins in the juice of succulent onion scales may have a rôle in restricting the advance of certain invading organisms (*6*).

The second group of toxic substances was found in the water extract from the dry outer colored-bulb scales from which the volatile substances had disappeared (*4*). Spores placed in extracts of sufficient concentration were either entirely prevented from germinating or, if germination started, the process was so modified as to cause the exudation of naked cytoplasm from the tip of the young germ tube, thus inactivating the organism. As the concentration of the extract was reduced, the toxic effects were proportionately decreased until eventually normal germination and growth occurred. In contrast to this, extracts from white scales showed little or no toxicity and supported good germination and growth. It appeared therefore that the natural resistance of colored onion bulbs to the smudge organism is based upon the toxic substances associated with the pigmentation of scales. It is presumed that in nature amounts of these toxins sufficient to inactivate the fungus (if present) dissolve into drops of meteoric or soil water which come in contact with colored scales. The outer colored scales therefore serve as a barrier to invasion by the parasite.

In the course of this investigation it became of interest to extend the same line of experimentation to a number of other fungi, especially those which are parasitic upon onion bulbs. The relation of onion oils to a number of organisms has been reported (*6*). The present paper deals with the relation of the pigment extracts to practically the same groups of fungi, namely, *Fusarium cepae* Hanzawa, *Fusarium* sp. No. 45 (an onion bulb-rotting organism), *Fusarium lycopersici* Sacc. (tomato wilt organism), and *Fusarium graminearum* Schwabe (*Gibberella saubinetii* (Mont.) Sacc.) (wheat scab organism); onion neckrot fungi, *Botrytis allii* Munn, *Botrytis* sp. No. 110, and *Botrytis* sp. No. 108a; *Aspergillus* sp. No. 4660 [3] and *Aspergillus niger* van Tiegh.; and an unidentified species of

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[2] Reference is made by number (italic) to "Literature cited," p. 514.

[3] As noted in another article (*6*), this species will readily decay onion bulbs. It is a yellow-spored form which is being studied by Dr. Charles Thom, Bureau of Chemistry, U. S. Department of Agriculture, and for the present is referred to by his number, 4660.

Helminthosporium which occurs on the dry outer scales of white onions. Varietal resistance to these groups is not uniform. No conspicuous resistance to the Fusaria has been observed (*7*). In the case of neckrot, however, the disease is more prevalent on the white than on colored varieties. *Aspergillus niger* is common on both colored and white varieties. *Helminthosporium* sp. has been found only on white varieties. The data secured for each group and their possible bearing upon paraistism and resistance will be considered separately.

METHODS OF EXPERIMENTATION

The method previously described was employed (*4*). Spores of the organism were suspended in sterile water, and equivalent drops of the suspension were transferred to glass slides in moist chambers. Uniformly white or colored, dry, outer scales were secured from White, Red, and Yellow Globe onions. These were cut into 2 mm. squares. Rapid diffusion of toxin into the liquid followed the addition of these fragments to the drops. To gain a gradation of concentration, one, two, and four fragments, respectively, were added to a series of three drops for each type of onion scale. This method, while obviously subject to some experimental error, gave the general comparison which was desired. Control drops were included in each series.

As will be shown presently, the toxicity of the pigmented extracts was quite general and the toxic effects were of the same general type as those noted with the smudge organism (*4*).

The effect on germination was exhibited either as complete inhibition or by the exudation of cytoplasm from the tip of the germ tube, usually shortly after the germ tube had formed. The latter reaction was not constant, however. It would seem that a very delicate balance in the concentration of the toxin is necessary to bring about the phenomenon. Too high a concentration of toxin evidently completely inhibits germination; too low a concentration allows the more normal hyphal development. At a certain point initiation of growth is permitted, but this is soon checked in such a way as to bring about the exudation of cytoplasm. The nature of this phenomenon is not entirely explained. It is suggested that the toxin retards or stops the wall-forming process at the tip of the germ tube, while imbibition of liquid by the spore protoplast continues. This leads to "rupturing" of the cell membrane at the weakest point followed by exudation of the cytoplasm. In certain cases the presence of a membrane around the exuding cytoplasm is suggested, but more often no such confining membrane can be distinguished.

When hyphal development does occur, the rate of growth is influenced by the concentration of the toxin. The most reliable criterion for comparison was found to be the percentage of normally germinating spores and the rate of germ-tube growth of the spores which germinated. These were estimated after a given interval by counting 100 or more spores and measuring the length of 40 or more germ tubes in each drop. As previously noted with *Colletotrichum circinans*, decrease in concentration of the toxin caused all the organisms studied to return gradually to normal germination and growth. Consequently the effects were not the result of lack of food but were due to the presence of inhibitory substances. In this report it has been considered sufficient to give the data secured from the exposure of the various organisms to the highest concentration of the toxins, that is, where four fragments of scale tissue were added to the drop.

RELATION OF PIGMENT TO DEVELOPMENT OF CERTAIN SPECIES OF FUSARIUM

Two strains of Fusarium which had been proved to be pathogenic to onion bulbs were used. One of these was isolated from bulbs collected in Illinois and agrees with the description of *Fusarium cepae* Hanzawa. Further studies of this disease are reported in another paper (*7*). The second strain was isolated from a decaying onion at Valencia, Spain. Since it has not as yet been identified with any previously described species, it is referred to in this paper as *Fusarium* sp. No. 45. Two species of the genus, nonpathogenic to onion, were included—one a vascular parasite of tomato, *Fusarium lycopersici* Sacc., and the other a parenchymatous invader of wheat and corn, *Fusarium graminearum* Schwabe (*Gibberella saubinetii* (Mont.) Sacc.). Tests with *Colletotrichum circinans* were included for comparison.

From the data given in Table I it will be seen that the addition of white scale tissue to the spore suspension did not materially affect the percentage of spore germination of any of the organisms studied, with the exception of the tomato Fusarium, in which case there was some reduction. The addi-

TABLE I.—*The effect of water extracts from dry outer scales of White, Yellow, and Red Globe varieties of onion on the germination and growth of several species of Fusarium as compared with Colletotrichum circinans*

Organism	Experiment No.	Germination in—				Length of germ tubes in—			
		Control	White scale extract	Yellow scale extract	Red scale extract	Control	White scale extract	Yellow scale extract	Red scale extract
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Microns*	*Microns*	*Microns*	*Microns*
Colletotrichum circinans	1	99	80	0	2	414	224	0	0
Fusarium cepae	1	95	95	0					
	2	87	98	8	6	582	368	134	17
F. sp. 45	1	100	95	23					
	2	50	50	1	0				
F. lycopersici	1	99	56	0	1	96	36	0	
F. graminearum	1	95	95	1	0				

tion of dry, outer, yellow or red scale tissue, however, resulted in a general reduction of germination or incomplete inhibition. The effects are quite as marked as earlier noted for *Colletotrichum circinans.* The growth of the germ tubes is somewhat reduced by the addition of white tissue in the case of both *Fusarium cepae* and *Colletotrichum circinans.* With *Fusarium cepae,* where some germination occurred in colored scale extract, marked retardation of growth is evident. As found with *Colletotrichum circinans,* no striking difference in the comparative toxicity of extracts from red and yellow scales was noted.

The data show that onion Fusaria are distinctly inhibited when subjected in this way to water extracts from dry, outer, colored scales. From the tests with two other species of Fusarium it may be expected that the toxins are equally effective upon forms other than those parasitic on onion bulbs. Should these organisms normally invade the bulb through the outer scales we might expect a difference between white and colored varieties in susceptibility, as generally noted for onion smudge. Since, however, the onion Fusaria normally invade through wounds or possibly through natural openings in the stem plate (7), the possibility of the scale toxins coming into immediate contact with the invading parasite is very slight. Consequently, little or no evidence of resistance to Fusarium bulb rot can be expected among colored varieties, and this supposition is confirmed by the writers' studies so far. In another paper (7) a field experiment is reported wherein plantings of several varieties of onion, including the three color types, were artificially inoculated with *Fusarium cepae.* Infection results at the end of the season were as follows:

	Percentage of fusarium bulb rot
Variety:	
Red Globe	12
Yellow Globe	8
White Globe	18
White Portugal	8

There is therefore no marked indication of varietal resistance between the colored and white varieties in this instance. Further study on this point is needed.

RELATION OF PIGMENT TO DEVELOPMENT OF CERTAIN SPECIES OF ASPERGILLUS AND HELMINTHOSPORIUM

Aspergillus niger is found commonly after harvest on the outer scales of onion bulbs, where it develops largely as a saprophyte under sufficiently moist conditions. Aggressive invasion of the fleshy scales is seldom if ever to be found. Although perhaps somewhat more common and conspicuous on white varieties, the colored forms are often affected as well. *Aspergillus* sp. 4660 was isolated from decaying garlic bulbs and by inoculation experiments has been shown to be an active parasite of the succulent scales of onion.

The data in Table II show that neither of these forms is inhibited by outer white scale extract. In the case of *Aspergillus* sp. 4660, germination was always better in the white scale extract than in the control; as a rule it was either decidedly retarded or entirely inhibited in the colored scale extracts. Likewise, growth in the white scale extract was about equal to that in the control, or better, while in the colored scale extracts it was decidedly retarded. In one experiment with *A. niger,* germination was inhibited by yellow scale extract and much reduced by the red scale ex-

tract. Where germination did occur, however, the growth was greater than in the control. In the second and third experiments the percentage of germination of *A. niger* was increased over the control in both white and colored scale extracts, and growth was practically the same in all three extracts.

It may be indicated, therefore, that whereas *Aspergillus* sp. 4660 is decidedly inhibited by the water extract from colored scales, *A. niger* is only moderately retarded, if at all. Hence no marked varietal resistance to the latter organisms would be expected in the colored types. This is in accord with the general observation.

TABLE II.—*The effect of water extracts from dry outer scales of White, Yellow, and Red Globe varieties of onion on the germination and growth of certain species of Aspergillus and Helminthosporium as compared with Colletotrichum circinans*

Organism	Experiment No.	Germination in—				Length of germ tubes in—			
		Control	White scale extract	Yellow scale extract	Red scale extract	Control	White scale extract	Yellow scale extract	Red scale extract
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Microns*	*Microns*	*Microns*	*Microns*
Colletotrichum circinans	1	99	80	0	2	414	224	0	0
Aspergillus niger	1	38	97	0	8	11	55	0	40
	2	49	90	90	100				
	3	0	89	88	52	0	203	190	195
A. sp. 4660	1	88	98	1	0	47	38	1	0
	2	42	80	3	0				
	3	6	97	2	0	21	85	10	0
Helminthosporium sp	1	84	100	0	2				

Helminthosporium sp. is a small-spored form which commonly causes dark-colored blotches on the outer scales of White Globe onions, but has never been noted on the colored forms. The toxic effect of colored scale extract is quite as distinct as with any other organism studied, while the white scale extract is apparently beneficial. It is probable, therefore, that the toxins in the red and yellow bulbs serve to restrict this organism largely to the white varieties.

RELATION OF PIGMENT TO DEVELOPMENT OF BOTRYTIS AND TO NECKROT RESISTANCE

Of the three species of Botrytis associated with the neckrot of onion, one, *Botrytis allii*, has been fully described by Munn (*1*). The remaining two species will be described in another paper by the senior writer, and will be referred to here as *Botrytis* sp. 110 and *Botrytis* sp. 108a. Although these forms commonly occur together, individually they cause somewhat different symptoms and have been respectively designated gray mold neckrot, mycelial neckrot, and small sclerotial neckrot. All three organisms are in the main wound parasites and usually enter through the neck of the bulb. The first two species are active producers of decay, commonly invading the entire bulb. The third form is less aggressive and is more often confined to the outer scales.

The marked resistance of colored varieties of onion to Botrytis has long been recognized by onion growers and dealers. The greater susceptibility of white forms to *B. allii* is noted by Munn (*1*). Selby also mentions this fact (*2*), although it is not clear with which species of Botrytis he deals, since he undoubtedly was in error in referring to the causal organism as *Sclerotium cepivorum*. *Botrytis* sp. 110 and *Botrytis* sp. 108a are the commonest in the Wisconsin and Illinois onion-growing sections, and the writers' critical field observations are confined largely to these forms. In 1920 and 1923 white, red, and yellow onions were planted on old onion soil at Racine, Wis. The crops were placed in storage and the development of mycelial neckrot during that period noted.

The White Globe variety developed 56 per cent of mycelial neckrot in 1920 and 44 per cent in 1923. The Red Globe variety developed less than 1 per cent in both periods. Yellow Globe developed 6 per cent in 1920, but was not included in the 1923 test. Yellow Strasburg, not included in the test of 1920, developed less than 1 per cent in 1923.

The marked resistance of the colored varieties is thus evident. In both of these experiments comparative susceptibility to *Botrytis* sp. 108a was also

noted. A high percentage of the white bulbs was infected with this form in both years, while in no case was the fungus found on normally pigmented bulbs of the colored varieties. In 1920 the Yellow Globe seed used was not of a pure strain. A small number of the bulbs formed very little yellow pigment. *B.* sp. 108a developed on these, illustrating further the close correlation between pigment and resistance.

In spite of this marked expression of resistance, it is not uncommon to find cases where a fairly large percentage of colored bulbs are decayed with neckrot. That the extent of resistance to neckrot is limited is further substantiated by experiments in which spores and mycelium of *Botrytis allii* and *Botrytis* sp. 110 were injected through needle wounds into the succulent neck tissue of white, red, and yellow bulbs. Under such conditions the organisms were only slightly, if at all, exposed to the water-extractable toxins in the dry outer scales. In all of these experiments both organisms caused colored and white bulbs to decay with apparently equal rapidity. It is evident, therefore, that the resistance of colored bulbs to *B. allii* and *B.* sp. 110 is a resistance to initial infection, and where this is not prevented and the fungus becomes established in the succulent tissue all forms are equally susceptible.

Data secured from a study of the effect of outer-scale extracts upon germination and growth are of interest at this point. A strain of *Botrytis cinerea* isolated from cyclamen and nonpathogenic to onion was included in these tests. Table III shows that exposure to white scale extract had no detrimental effect on any of the forms tested; and in some instances the result was beneficial. In the case of yellow and red scale extracts the toxic effects are quite as marked as already noted for Fusarium, Aspergillus, Helminthosporium, and Colletotrichum. In two experiments with *B.* sp. 110 a high percentage of germination occurred in yellow scale extract, but it is to be noted that very meager growth resulted in these cases.

From the data just cited, resistance of colored bulbs is to be expected where the organisms are exposed to the toxins present in the outer colored scales. To what extent does this correlation exist in nature? The three forms of Botrytis parasitic on onion do not ordinarily attack the growing plants, but subsist as saprophytes until harvest time. As the tops of the onion mature, entrance is gained through the "neck" of the bulb. Comparable lots of White Globe bulbs, with the tops removed from one lot and left intact in the other, showed that in an appropriate environment the infection occurs as readily through an intact neck as through a wounded one. Therefore as the top dies down these organisms may be expected to invade the dying tissue at the neck and gradually enter the succulent tissue of the bulb. A considerable amount of moisture is necessary for growth at this point, as the senior writer has demonstrated (*5*) that *Botrytis* sp. 110, at least, can be almost completely checked by artificial drying of the neck tissues after incipient infection has taken place. In the necks of colored bulbs at this stage of maturity there is ordinarily a considerable quantity of intensely colored dead leaf or scale tissue. The extreme outer scales of colored bulbs usually develop little or no pigment at the

TABLE III.—*The effect of water extracts from dry outer scales of White, Yellow, and Red Globe varieties of onion on the germination and growth of several species of Botrytis as compared with Colletotrichum circinans*

Organism	Experiment No.	Germination in—				Length of germ tubes in—			
		Control	White scale extract	Yellow scale extract	Red scale extract	Control	White scale extract	Yellow scale extract	Red scale extract
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Microns*	*Microns*	*Microns*	*Microns*
Colletotrichum circinans	1	99	80	0	2	414	224	0	0
Botrytis allii	1	64	55	0	0	280	294	0	0
	2	97	94	4	4	491	554	5	28
	3	49	55	0	0				
B. sp. 110	1	53	99	1	0	24	209	5	0
	2	53	100	94	0	24	82	10	0
	3	90	97	0	0				
	4	90	100	93	10				
B. sp. 108a	1	100	100	4	14	341	839	27	39
	2	72	90	8	2	125	394	15	8
B. cinerea	1	93	96	0	3	143	166	0	7

neck and, as already noted (*4*), these are commonly affected with smudge during the latter part of the growing season. Nevertheless, some pigment develops in the underlying neck scales, and it becomes more intense with the desiccation of these tissues at maturity. The neckrot fungi entering at this latter period are thus exposed to the soluble toxins of dry colored scales, from which it is certain that the soluble toxins readily diffuse into any drops of moisture present.

It is believed that at this point the toxins associated with the scale pigments become the principal factor of resistance to Botrytis and serve as a barrier to infection. It is obvious that conditions are not always favorable to the expression of this resistant character. Frequently bulbs are harvested and the tops removed before complete maturity. This condition is unfavorable to the exclusion of the neckrot fungi, since it leaves fewer dry colored scales on the necks of colored bulbs and exposes a greater amount of succulent wound tissue, which is most conducive to the establishment of the fungus.

From this analysis of the question it may be assumed that the colored varieties of onion possess a quality of resistance to the three species of Botrytis causing the various types of neckrot. This resistance functions as a barrier to initial establishment of the fungi in the succulent tissues. Certain conditions of environment and handling of the crop may be expected to overthrow this means of defense at times. Resistance, therefore, in this case, as in most others, is not to be considered as absolute. There is, however, a definite substance (or group of substances) associated with pigmentation of the bulbs of colored varieties of onion which is toxic to the neckrot fungi, and this accounts in the main for the reputed resistance of these types to neckrot as compared with the well-known susceptibility of white varieties.

DISCUSSION OF RESULTS

Certain outstanding facts in the investigation to date of disease resistance in the onion may well be reviewed at this point. There exist in the onion tissue substances which, when extracted, exhibit a surprising amount of toxicity not only to fungi nonpathogenic to onion and to its mild parasites, but to its more aggressive parasites as well. Yet a fungus parasite is frequently found invading the tissue of the onion bulb, often in close proximity to these toxic substances or to substances from which the toxins are rapidly formed upon injury to the tissues. Therefore the mere presence of toxic substances in the host tissue does not necessarily imply resistance. The avenue of invasion and the device by which the organism parasitizes the host cell influence the ultimate effect which toxic substances within this cell may have upon the invader.

The studies on volatile onion oil (*6*) show that the toxicity in this case is general rather than specific to a given organism. Although the more rapidly invading bulb parasites are as a rule less sensitive to the volatile toxins than are the milder parasites, they do not ordinarily germinate or grow in the extracted onion juice, and are retarded materially by the volatile toxins from undiluted juice. Moreover, there is no correlation between the conspicuous resistance of colored varieties to smudge and neckrot and the amount of volatile toxins in the tissue. While it is postulated that the different degrees of retarding effect of the volatile toxins upon the different organisms may be responsible in part for the variation in parasitism of these organisms, this variation may be influenced by other factors as well, such as (1) mode of entrance, (2) method of attacking the host cell, (3) advance lethal effects of fungus enzymes, (4) food elements in the host.

The study of the range of toxicity of outer colored scale extract shows that this substance, as in the case of the volatile oil, is quite generally toxic to the fungi. With one notable exception (*Aspergillus niger*), the germination and growth of all of the organisms tested (those pathogenic and those nonpathogenic to onion) are decidedly inhibited in colored scale extracts, while they thrive in the extract of white scales. But here again the correlation between color and resistance is not general for all the bulb diseases of onion.

It would appear from the present studies that the toxic substance in colored scales becomes functional as a repellent principle in the dry outer bulb scales. There it diffuses readily into soil or meteoric water and inactivates the fungus, preventing its entrance into the living tissue of the underlying succulent scales. Consequently, those parasites which by nature of their respective channels of invasion come into direct contact with this toxic substance upon attacking the onion bulb would be expected to appear less frequently on colored than on white varieties.

This is strikingly true in the case of smudge (*Colletotrichum circinans*), where the parasite normally attacks at any point on the scale surface. In the case of black mold (*Aspergillus niger*) there is slight if any correlation between color and resistance. The organism attacks by almost precisely the same avenue as smudge, but the writers' tests have shown that the colored extracts have no consistent toxic effect upon this fungus. In the case of neckrot the entrance is usually through the neck of the bulb at about harvest time. Contacts with the toxins may be expected in a majority of cases, although as pointed out, this status may be changed under some conditions. Field observations show that there is a strong correlation between color and resistance to neckrot, but probably it is not so general as in the case of smudge.

Helminthosporium sp., which is essentially a saprophyte capable of attacking only the dead outer scales of onion bulb, has been found only on white varieties, but little is known as to its general occurrence. From its sensitiveness to the colored scale extract it is likely to be limited largely to the white varieties. There are probably other saprophytic soil organisms which occasionally develop on the outer scales of white varieties and which under certain conditions may act as mild parasites of the bulb in storage, but from which colored bulbs are protected by the repellent toxins in the outer scale.

The Fusarium bulb-rot organisms which invade most commonly through the stem plate, or through insect wounds, probably come under little or no influence of the toxins from the outer colored scales at the point of infection. Though they are very sensitive to these toxins, a strong expression of disease resistance would not logically be expected in this case. The limited evidence at hand points in this direction.

SUMMARY

(1) The marked resistance of colored types of onions to smudge (*Colletotrichum circinans*) is due to the presence in the outer scales of certain toxic substances closely associated with the pigment compounds or identical with them. The functioning of this resistant principle depends upon the ready diffusibility of these toxins from the dead outer scale into drops of meteoric or soil water, where they inactivate the fungus before it can attack the onion tissue. The purpose of the present investigation has been to determine the effect of these soluble toxins on certain other fungi, especially those which attack onion bulbs.

(2) The fungi considered, besides the smudge organism, were two onion-bulb-rotting species of Fusarium, namely, *F. cepae* Hanzawa and an unidentified form, referred to as *Fusarium* sp. 45; the tomato-wilt organism, *Fusarium lycopersici;* the wheat-scab organism, *Gibberella saubinetii* (*Fusarium graminearum*); the onion black-mold organism, *Aspergillus niger;* a garlic and onion bulb-decaying organism, *Aspergillus* sp. 4660; *Helminthosporium* sp. which occurs on the outer scales of white varieties; and three forms of *Botrytis allii*, Botrytis associated with onion neckrot, *Botrytis* sp. 110, and *Botrytis* sp. 108a.

(3) With one exception, *Aspergillus niger* (see below), the organisms germinated and grew quite as well in the extracts from dry outer white scales as in the control drops; but when extracts from dry outer colored scales were used, germination and growth were greatly retarded or entirely inhibited.

(4) The function of these toxins in rendering the bulb resistant to a given organism depends upon the organism's mode of attack.

(5) In the case of the onion Fusaria, which enter through insect wounds or through openings in the stem plate, there is little chance of contact with the soluble toxins in the outer scales. No evidence of varietal resistance correlated with pigmentation has been noted in this instance.

(6) *Aspergillus niger*, which normally attacks the outer scales, and which seems to be little affected by the outer scale toxins, develops quite as well on colored as on white varieties.

Helminthosporium sp., which is sensitive to the toxins, is evidently limited or restricted largely to the outer scales of white varieties.

(7) The neckrot fungi by their mode of entrance through the neck tissues ordinarily encounter in colored varieties a certain amount of the outer scale toxins. The generally observed escape of colored varieties from neckrot is probably due to this factor. More or less neckrot on colored varieties does occur in nature, however. Experimentally, it was shown that upon inoculation with *Botrytis allii* and *Botrytis* sp. 110 directly into the succulent tissue (thus eliminating the influence of the soluble toxins in the dry outer scales) infection occurred with approximately equal rapidity in colored and in white bulbs. It would appear therefore that the explanation for the occasional occurrence of neckrot epiphytotics in colored varieties will

be found in a sequence of particular conditions at harvest. One such condition is premature topping, which through wounds may expose succulent tissue to parasitic attack.

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ASEXUAL PROPAGATION AS AN AID TO THE BREEDING OF ROOTSTOCKS [1]

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It has been stated [2] that—

> nearly all plants may be propagated by cuttings from one or another of their parts. The ease with which plants may be multiplied in this way varies greatly in different species, and even in different varieties of the same species.
>
> Climate exerts a marked influence upon the tendency of plants to develop from cuttings. In certain localities in southern Europe and in parts of South America branches of the common apple tree, sharpened and driven into the ground as stakes, often take root and sometimes even bear fruit during the same season.

A comparatively warm soil and a cool atmosphere with abundant soil moisture are favorable to the rooting of cuttings. A coarse, sharp, clean sand has been recommended as the best material for use indoors, since it provides ample drainage and is comparatively free from the damping-off fungus. For cuttings which form roots readily, a mixture of one part of light garden loam to two parts of sand may be used. The cuttings should be kept in an environment sufficiently moist to prevent loss of water by evaporation and sufficiently warm to favor moderate root growth. The tops should be kept cool enough to prevent the early growth of leaves.

Cuttings of larger diameter root more readily than those of smaller diameter, which are more apt to shrivel and die. Wounds on the lower end of the cuttings start to heal by the production of a loose cellular mass of tissue known as a callus. The roots do not arise from the callus itself but from internal tissue. In many plants the roots bear no relation to the callus in position, as, for instance, in the figure shown in Plate 1, Group 3, C, where the roots are seen arising from the bark in the middle of the cutting. Bailey [3] states, however, that "as a matter of practice, best results are obtained from callused cuttings, particularly if the cuttings are made from mature wood, but this is probably due to the fact that considerable time is required for the formation of adventitious buds which give rise to the roots, not to any connection between the callusing and rooting processes themselves."

It has been the purpose of the preceding paragraphs to review briefly a few of the principles of the propagation of plants from cuttings in order that the results reported in this paper may be interpreted to better advantage. Let us now turn to a brief consideration of the desirability of asexual propagation of rootstocks from the standpoint of the breeding of improved types of pomological plants.

The breeding of improved pomological varieties has occupied the attention of horticulturists for a number of years. Most of this breeding work is of value for the number of varieties which have been produced rather than for an analysis of hereditary characters, as was pointed out by the writer in 1923.[4] This condition has resulted from the fact that long-life-cycled plants do not meet the requirements of favorable genetic material discussed by Babcock,[5] Malloch,[6] and others. The breeding of horticultural forms has been largely directed toward the improvement of the scion wood rather than of the rootstock. As soon as a desirable new type has been discovered, it has been propagated by asexual means, thus utilizing immediately the favorable morphological variations as well as any increased physiological vigor which the new type may possess. The breeding of scion wood has proceeded along these lines, however desirable a genetic analysis might be from the standpoint of future improvement.

The breeding of improved types of rootstocks has not received the attention it deserves, owing partly to the increased difficulty and expense of conducting such investigations and partly to the less obvious value of such endeavors. The more thoroughgoing

[1] Received for publication June 20, 1924—issued February, 1925.

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horticulturists recognize, however, the importance of the rootstock in its relation to soil conditions, to disease resistance, to uniformity of growth, and to the manner of union with the scion. In breeding for disease resistance it frequently happens that the resistance sought for resides in a distinct species. Such a species may or may not graft readily with the commercial type which it is desired to grow. It might be possible to unite the qualities of disease resistance and a possibility of grafting by hybridization. Such a hybrid would of course be heterozygous and would segregate in succeeding generations. In order to produce a desirable type for the propagation of rootstocks from seed it would be necessary to purify such a hybrid in succeeding generations. Such a procedure is costly both in time and resources. Add to this the possibility of sterility in the species cross, and the desirability of asexual propagation becomes more and more obvious.

Even when breeding is not for disease resistance, uniformity is desirable. In propagating rootstocks from seed, uniformity demands a homozygous parent tree. Relative homozygosity may be obtained in fruit trees with considerable expenditures, but at the present time our knowledge as to the purity of different forms is rather meager.

Hybrid vigor is utilized to considerable advantage by corn growers and it should be of as great advantage to pomologists. To utilize hybrid vigor of the first generation in fruit trees it is necessary to be able to propagate the hybrid by asexual means.

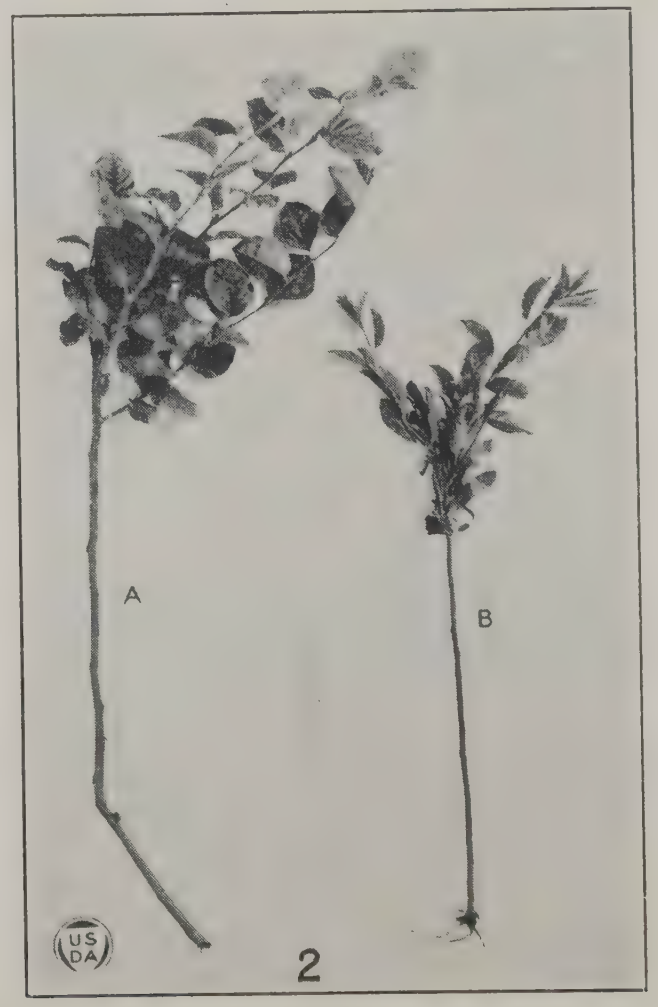
In order to test the relative merits of new types of scion wood derived from seedlings, they should be given uniform environmental conditions. Such uniformity as to rootstocks could be best secured by asexual propagation of the rootstock. Perhaps such a critical study of scion wood derived from seedlings which generally show great variability would not interest the practical breeder, but what about bud variations? Geneticists recognize two kinds of bud variations—first, modifications, and second, mutations. Modifications are ever present in fruit trees and are usually due to differences in combinations of environmental conditions, either internal or external, which existed during the development of that particular organ. Bud mutations, while comparatively rare, do occur in a large number of plants. Bud mutations are transmissible, while modifications are not. In order to determine whether we are dealing with a bud mutation or a modification, except in very striking cases, such as the production of red plums on the branch of a yellow plum tree, we must test out the variation to see whether or not it will come true to type. Such a test demands uniform rootstocks best secured by asexual propagation.

Cuttings of a large number of species and varieties were planted in February, 1923, to ascertain how many would root readily from cuttings. It was necessary to terminate the experiment in May, 1923. The cuttings were 1 foot long and from three-fifths to one-half inch in diameter. They were planted 10 inches deep, leaving about 2 inches exposed. The soil was a light loam mixed with a greenhouse soil consisting of peat, leaf mold, sand, manure, and loam. The soil was kept moist throughout the course of the experiment which was conducted on a well-drained piece of land in Berkeley, Calif. The cuttings were obtained from the University Farm, Davis, Calif., the United States Plant Introduction Gardens, Chico, Calif., the Citrus Experiment Station, Riverside, Calif., and the Connecticut Agricultural Experiment Station, Storrs, Conn. The accompanying table lists the number of cuttings of each sample which formed leaves, shoots, callus, roots, or made no growth. Some of the cuttings formed either a callus or roots in all of the forms listed in the table. A summary is given at the end of the table showing the species and varieties which failed to form either a callus or roots under the particular conditions of the experiment.

From the review given in the first part of the paper and from the description of the conditions of the experiment, the reader may conclude that experimental conditions were not

EXPLANATORY LEGEND FOR PLATE 1

Group 1:
A.—Rooted cuttings of *Prunus besseyi* from Connecticut.
B.—*Prunus munsoniana* from Connecticut.
C.—*Prunus pumila* from Connecticut.
Group 2:
A.—Rooted cuttings of *Cydonia oblonga*, SPI 33214.
B.—*Prunus bokhariensis*, SPI 40229.
Group 3:
A.—Rooted cuttings of Satsuma plum, Davis 13–12.
B.—Clyman plum, Davis 5–9.
C.—Hamari fig, SPI 6468.
D.—F_2 Strawberry × Peento, peach hybrid Davis, 1–12



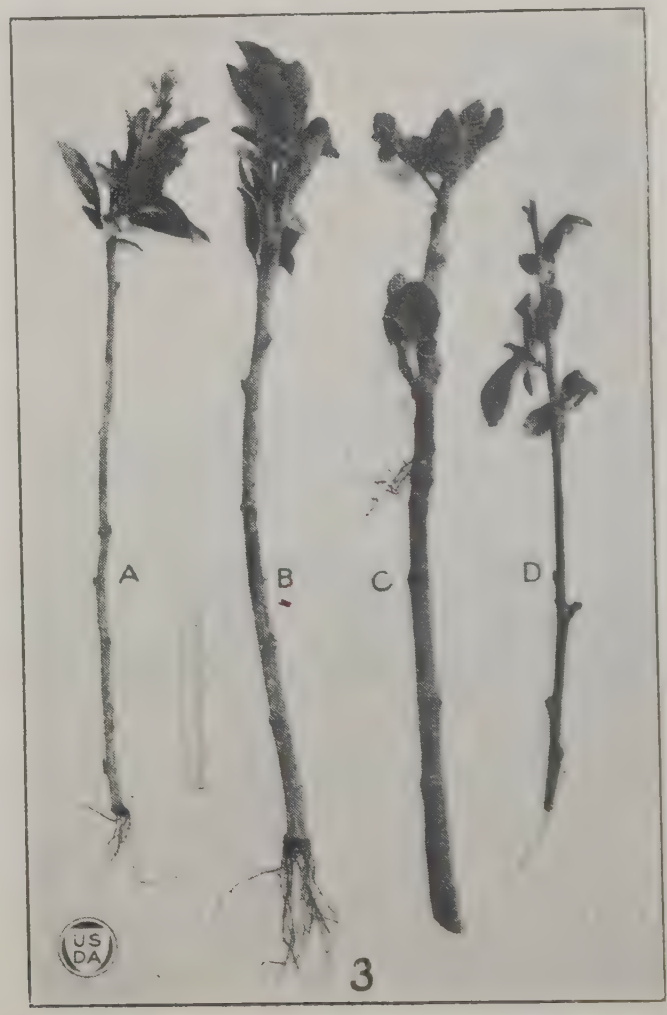


PLATE I

A sexual Propagation in the Breeding of Rootstocks

(For explanatory legend see p. 516)

ideal in every respect for the rooting of cuttings. The soil, although well drained, would not, of course, be comparable to sand in this respect. It was not possible to supply bottom heat for the roots or to control atmospheric temperature. A number of the cuttings put forth leaves before a callus had formed, thus increasing transpiration. In view of these conditions the cuttings which did root are all the more interesting as examples of species or varieties which will root under slightly unfavorable environmental conditions. Under proper experimental conditions it might be possible to utilize asexual propagation to a far greater extent than is realized at present. It is with this thought in mind that the writer has submitted the data collected, hoping that others may continue the study beyond the point which has been possible in the present experiment.

After it has been demonstrated that a particular form will root readily from cuttings, it remains to be shown that such a rooted cutting will continue to develop and grow normally. The writer has not been able to continue the experiment to this stage. It is then necessary to select the particular form suited to the scion, to the soil, and to other environmental conditions. The Marianna type is known to root from cuttings, but it is open to the objection that it suckers freely in certain sections of the country. The Myrobalan plum is extensively used as a stock for different varieties of plums and prunes and can be grown from cuttings. There are several different types of Myrobalan plums and these will hybridize with certain other plums. Such hybrid seeds would probably cause a considerable variation in the resulting seedlings. The propagation of a desirable type of Myrobalan plum by asexual means would be a practical method of securing uniformity and a direct application of the ideas which the writer has tried to set forth in this paper. It is interesting to note that some nurserymen are actually doing this in California. It is hoped that the data presented in the accompanying table may be of some service to future investigators.

TABLE I.—*Showing data for the species and varieties of cuttings which formed either roots or a callus*[a]

Species and varieties	Source	Cutting No.	Leaves	Shoots	Callus	Roots	No growth
Chaenomeles lagenaria cathayensis	Chico	SPI 37954		3	3		
Do	do	SPI 46129	4		2		
Cydonia oblonga	do	SPI 32882		5	5		
Do	do	SPI 33214		5		5	
Ficus carica	do	PLH 6243	3			1	1
Archipel	do	SPI 18835	1			1	4
Constantine	do	SPI 18874	1		1		4
Hamari	do	SPI 6468	3			3	3
Maslin No. 20	do		2			1	3
Oeil de Perdrix	do	SPI 18842	1			1	4
Reculver	do	SPI 18868	2			2	3
Warren	do	SPI 18905	1			1	4
Xehba	do	SPI 6941	7			2	
Prunus amygdalus	do	SPI 7398	5		5		
Do	do	SPI 26543		3	3		
Do	do	SPI 33217	3	6	5		
Prunus sp. Buckthorn almond	do	SPI 28942		4	4		
Prunus persica	do	SPI 33219	7		1		
Do	do	SPI 36703	6		1		
Do	do	SPI 38469	4		4		
Do	do	SPI 40900		1	1		3
Do	do	SPI 43130	10		4		
Do	do	SPI 43133	10		4		
Do	do	SPI 43289	10		4		
Do	do	SPI 55564	8		8		3
Do	do	SPI 55835	10		4		
F_2 Strawberry × Peento	Davis	1–12	1			1	9
Family Favorite × Kalamazoo	do	1–4	1		1		9
Prunus persica nucipersica	Chico	SPI 29227	7		5		
Do	do	SPI 34685	10		4		
Do	do	SPI 43141	11		3	1	
Do	do	SPI 43142	10		1		
Do	do	SPI 43144	9		7		
Prunus fenzliana	do	SPI 35205	6		1		
Do	do	SPI 43303	8		1		
Prunus besseyi	Conn		7	18	10	8	
Prunus pumila	do			19	12	7	
Do	Chico	PIG 19261		3		3	
Do	Riverside		16			16	
Prunus sp. Gigantic plum	Chico			3	3		
Prunus sp. Discovery plum	do			2	1	1	
Prunus sp. Methley	do	SPI 31652		10	10		
Prunus sp	do	SPI 32751	3	1	1		
Prunus sp. (Alpha)	do	SPI 43176		3	3		

TABLE I.—*Showing data for the species and varieties of cuttings which formed either roots or a callus*[a]—Continued

Species and varieties	Source	Cutting No.	Leaves	Shoots	Callus	Roots	No growth
Prunus sp. (Sharp Early)	Chico	SPI 43178		4	4		
Prunus sp. (Wright Purple)	do	SPI 43181		7	7		
Prunus sp. (Best Hybrid)	do	SPI 43182		14	12		
Prunus sp	do	SPI 47935	4		1		
Prunus armeniaca	do	SPI 26048	4		1		
Do	do	SPI 28961	3		1		
Do	do	SPI 28958	3		1		
Do	do	SPI 28959	2		2		
Do	do	SPI 28960	4		1		
Prunus bokhariensis	do	SPI 40231		4	4		
Do	do	SPI 40233	4		4		
Do	do	SPI 40229		4		3	
Do	do	SPI 40224		1	1		2
Prunus cerasifera (purple)	Riverside.		20		10	8	
Prunus cerasifera	do		18		7		
Prunus cerasifera divaricata	Chico.	SPI 37463		3	3		
Prunus cerasifera × *P. hortulana* (?) (The Marianna type).	Riverside.			16		16	
Prunus dasycarpa	Chico			6	6		
Prunus domestica	do	SPI 30690		5	5		
Do	do	SPI 33224		4	4		
Do	do	SPI 30692		4	1		
Do	do	SPI 34268		7		7	
Prunus domestica (stock of Tribble Bros., Elk Grove, Calif.).	Riverside.		15		15		
Agen (French Prune)	Chico			2	2		2
Do	Davis	8–13	10		5		
Clyman	do	5–9	7		2	5	3
Columbia	do	9–9	10		2		
Grand Duke	do	12–9	10		8		
Imperial Epineuse	do	4–15	10		2		
Italian Prune (Fellenberg)	Chico.			5	1		
Peach Plum	Davis	13–8	8		6	1	1
Pond	do	11–9	10		3	2	
Sergeant	do	5–15	10		10		
Standard	Chico			5	5		
Sugar	do			4	4		
Do	Davis	2–15	10		10		
Sultan	do	12–13	2			1	4
Tragedy	do	8–9	8		1		2
Yellow Egg	do	7–10	10		2		
Prunus fremonti × (*Prunus cerasifera* ?)	Riverside.			19		19	
Prunus lycioides	Chico.	SPI 24808	4		4		
Do	do	SPI 28943	3		3		
Prunus mexicana	Conn.			27	27		
Do	Riverside.		32		17	1	
Prunus munsoniana	Conn.			22	21	1	
Prunus spinosa	Riverside.		12		2	2	
Prunus spinosa × *P. domestica*	Chico.	SPI 32671		9	4		1
Do	do	SPI 32673		10	10		
Prunus salicina:							
Abundance	Davis.	14–8	10		4		
Satsuma	do	13–12	10			10	
Santa Rosa	Chico.			3	3		
Prunus salicina (?) × Combination	Davis	3–9	9		4	5	
Prunus salicina × *P. armeniaca* (Rutland Plumcot).	Chico.			4		2	
Prunus salicina × *P. simonii*							
Wickson	Chico.			1	1		3
Climax	Davis.	15–19	10		4		
Prunus subcordata	Chico.	SPI 32168	4		2		
Pyrus sp	do	SPI 26591		5	5		
Do	do	SPI 30308	3	1	1		
Do	do	SPI 37071		5	5		
Do	do	SPI 38799		4	1		
Do	do	SPI 44276		4	2		
Do	do	SPI 43754		5	5		
Pyrus amygdaliformis	do	SPI 21982		5	5		
Pyrus betulaefolia	do	SPI 44006		6	6		
Pyrus calleryana	do	SPI 32736		4	4		
Pyrus communis	do	SPI 32739		4	4		
Do	do	SPI 32745		4	4		
Do	do	SPI 32746		6	2		
Do	do	SPI 47093		3	3		
Do	do	SPI 33207		7	3		
Favorita	Davis	20–34	10		6		
Anjou	do	20–20	10		8		
Bartlett	do	20–26	10		6		
Bloodgood	do	20–29	10		8		
Clairgeau	do	20–15	10		5		
Clapp Favorite	do	20–43	9		7		
Colonel Wilder	do	20–14	10		3		
Colorado Seedless	do	3–48	10		2		
Comice	Chico			3	3		
Dana Hovey	Davis	20–5	9		9		
Do							

TABLE I.—*Showing data for the species and varieties of cuttings which formed either roots or a callus*[a]—Continued

Species and varieties	Source	Cutting No.	Leaves	Shoots	Callus	Roots	No growth
Pyrus communis—Continued.							
Doyenne d'Alençon	Davis	20-31	9		5		
Easter Beurre	do	4-48	10		10		
Flemish Beauty	do	20-48	10		4		
Forelle	do	20-35	9		3		
Giffard	do	20-13	10		5		
Glou Morceau	do	20-28	10		10		
Hardy	do	20-19	10		7		
Do	do	20-40	10		4		
Howell	Chico			4	4		
Fox	Davis	20-52	8		5		
Kieffer	do	20- 4	10		8		
Lawson (Comet)	do	20-25	9		8		
P. Barry	do	20-41	10		6		
Seckel	do	20-37	10		3		
Summer Doyenne	do	20- 1	10		4		
Surprise	Chico	SPI 45901	2		2		
Winter Nelis	Davis	5-50	10		5		
Do	do	20- 9	10		6		
Pyrus nivalis	Chico	SPI 27670	3		1		
Pyrus phaeocarpa	do	SPI 39540	2	2	2		
Pyrus salicifolia	do	SPI 26680		3	3		
Pyrus serotina	do	SPI 30329		5		5	
Do	do	SPI 30352		5	5		
Do	do	SPI 30360		4	4		
Do	do	SPI 30361		4		2	
Do	do	SPI 38241		4	4		
Do	do	SPI 38242		3	3		
Do	do	SPI 38264	2	1	1		
Do	do	SPI 38271		5	1		
P. serotina × *P. communis*	do	SPI 43442		3		3	
Do	do	SPI 43443		5	3		
Do	do	SPI 43444		4	4		
Do	do	SPI 43562		4	4		
P. serotina (probably Golden Russet) × *P. communis.*	do	SPI 55805		4		4	
Pyrus ussuriensis	do	SPI 40019		6	6		
Do	do	SPI 44151		3	3		
Do	do	SPI 44150		5	5		
Pyrus sp. (Flowering crab)	do	SPI 22434		3	3		
Pyrus sp. (Muzalma)	do	SPI 30326	5		4		
Do	do	SPI 30326		5	2		
Do	do	SPI 30353		11	11		
Pyrus sp.	do	SPI 30635	5		2		
Do	do	SPI 30327	9		4		
Do	do	SPI 40207	6		6		
P. baccata × *P. malus*	do	SPI 28489		6	3		
Do	do	SPI 28489	4		1		
Pyrus malus	do	SPI 27152	4		1		
Do	do	SPI 27153	8		8		
Do	do	SPI 35636	3		1		
Do	do	SPI 39829	3		2		
Do	do	SPI 43154	2		1		
Do	do	SPI 43155	3		1		
Do	do	SPI 43157	17		9		
Do	do	SPI 43164	3		1		
Do	do	SPI 43168	3		2		
Do	do	SPI 43171	1		1		
Do	do	SPI 43173	3		2		
Pyrus malus:							
Chenango	Davis	8-44	10		10		
Cliff	Chico	SPI 43153	4		4		
Diadem	do	SPI 43157	4		1		
Early Harvest	Davis	7-45	10		10		
Gen. Carrington	Chico	SPI 43159	3		1		
Gravenstein	Davis	5-45	10		7		
John Sharp	Chico	SPI 43162	4		2		
Keswick	Davis	4-45	10		6		
Maiden Blush	do	8-44	10		8		
Red Astrachan	do	2-44	10		10		
Red June	do	9-45	10		1		
Red Spy	Chico	SPI 43169	4		2		
Summer Pearmain	Davis	10-45	10		7		
White Astrachan	do	1-48	10		10		
Willie Sharp	Chico	SPI 43174	3		3		
Yellow Transparent	Davis	2-50	10		3		
Pyrus pulcherrima (*P. floribunda*)	Chico	PIG 16625		7	7		
Pyrus sieboldi arborescens	do	SPI 43704		5	3		
Pyrus zumi	do	SPI 43705		6	6		3

[a] SPI refers to Seed and Plant Introduction numbers; PIG to Plant Introduction Garden numbers; and PLH to Plant Life History numbers.

The following failed to form either callus or roots: *Chaenomeles lagenaria cathayensis*, 2 varieties; *Crataegus pinnatifida*, 1 variety; *Ficus carica*, 79 varieties; *Prunus amygdalus*, 28 varieties; *P. persica*, 54 varieties; *P. persica* × *P. persica nucipersica*, 2 crosses; *P. persica nucipersica*, 5 varieties; *P.* subgenus *Amygdalus*, 8 species; *P. armeniaca*, 32 varieties; *P. avium*, 5 varieties; *P. cerasus*, 1 variety; *P. virginiana*, 3 varieties; *P. domestica*, 5 varieties; *P. salicina*, 4 varieties; *Prunus*, subgenus *Prunophora*, 23 species, 12 undetermined species; *Pyrus*, subgenus *Malus*, 5 species, 9 undetermined species; *P. malus*, 6 varieties; *P.* subgenus *Pyrus*, 2 species, 9 undetermined species; *P. serotina*, 13 varieties; *P. communis*, 8 varieties.

SUMMARY OF RESULTS

Species which formed roots and a callus: *Cydonia oblonga*, *Prunus besseyi*, *P. pumila*, *P. bokhariensis*, *P. cerasifera*, *P. fremonti* × *P. cerasifera* (?), *P. cerasifera* × *P. hortulana* (The Marianna type), *P. mexicana*, *P. munsoniana*, *P. spinosa*, *Prunus* sp. Discovery plum, *Pyrus serotina*, *P. serotina* × *P. communis*.

Species which formed a callus only: *Chaenomeles lagenaria cathayensis*, *Prunus fenzliana*, *P. dasycarpa*, *P. spinosa* × *P. domestica*, *P. lycioides*, *P. subcordata*, the following unnamed forms of Prunus, Gigantic plum, Methley, Alpha, Sharp Early, Wright Purple, Best Hybrid, SPI Nos. 32751, 47935; *Pyrus amygdaliformis*, *P. betulaefolia*, *P. calleryana*, *P. nivalis*, *P. phaeocarpa*, *P. salicifolia*, *P. ussuriensis;* the following unnamed forms of pears, SPI Nos. 26591, 30308, 37071, 38799, 44276; *Pyrus baccata* × *P. malus*, *P. pulcherrima*, *P. sieboldi arborescens*, *P. zumi*; and the following unnamed forms of apples, SPI Nos. 22434, 30326, 30353, 30635, 30327, 40207.

The following results were secured with commercial forms:

Varieties of *Ficus carica* which rooted: SPI 6243, Archipel, Hamari, Maslin No. 20 Oeil de Perdrix, Reculver, Warren, Xehba. Varieties of *Ficus carica* which formed a callus only: Constantine.

Varieties of *Prunus amygdalus* which formed a callus: SPI Nos. 7398, 26543, 33217, and *Prunus* sp. SPI No. 28942.

Varieties of *Prunus persica* which formed roots: F_2 Strawberry × Peento, *P. persica nucipersica* SPI 43141. Varieties of *P. persica* which formed a callus only, Family Favorite × Kalamazoo, SPI Nos. 33219, 36703, 38469, 40900, 43130, 43133, 43289, 55564, 55835; *P. persica nucipersica* SPI Nos. 29227, 34685, 43142, 43144.

Varieties of *Prunus armeniaca* which produced a callus only: SPI Nos. 26048, 28958, 28959, 28960, 28961.

Varieties of *Prunus domestica* which produced roots: SPI No. 34268, Clyman, Peach Plum, Pond, and Sultan. Varieties of *P. domestica* which produced a callus only: *P. domestica* stock of Tribble Bros., SPI Nos. 30690, 30692, 33224, Columbia, Italian Prune (Fellenberg), Agen (French Prune), Grand Duke, Imperial Épineuse, Sergeant, Standard, Sugar, Tragedy, Yellow Egg.

Varieties of *Prunus salicina* which produced roots: Satsuma, Combination and Rutland Plumcot. Varieties of *P. salicina* which produced a callus only: Abundance, Santa Rosa, Wickson and Climax.

Varieties of *Pyrus communis* which formed a callus only: SPI Nos. 32736, 32739, 32745, 32746, 47093, Favorita, Anjou, Bartlett, Bloodgood, Fox, Clairgeau, Clapp Favorite, Colonel Wilder, Colorado Seedless, Lawson (Comet), Comice, Dana Hovey, Doyenne d'Alençon, Summer Doyenne, Easter Beurre, Flemish Beauty, Forelle, Giffard, Glou Morceau, Hardy, Howell, Kieffer, P. Barry, Seckel, Surprise, Winter Nelis.

Varieties of *Pyrus malus* which formed a callus only: Chenango, Cliff, Diadem, Early Harvest, General Carrington, Gravenstein, John Sharp, Keswick, Maiden Blush, Red Astrachan, Red June, Red Spy, Summer Pearmain, White Astrachan, Willie Sharp, Yellow Transparent, and three plant introductions of the United States Department of Agriculture as follows: SPI Nos. 27152, 27153, 35636, 43154, 43155, 43157, 43164, 43168, 43171, 43173.

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THE DIAGNOSIS OF DECAY IN WOOD [1]

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INTRODUCTION

There are many industrial problems connected with the staining and rotting of wood. The questions asked by practical lumbermen could be answered much more satisfactorily if a reliable means were at hand for identifying the fungi producing decay in wood and wood products. The present study is an attempt to furnish a sound basis for future investigations leading toward more accurate diagnosis.

Although considerable work has been done by various investigators on many phases of the wood-rot problem, there is no literature dealing specifically with the diagnosis of decay. Early works on forest pathology were primarily histological studies, with a background of field observations on the life histories of the causal organisms. Both Theodor Hartig (*27*),[2] who as early as 1833 sought the cause of wood rots, and Willkomm (*90*), whose work appeared in 1866, believed in the spontaneous generation of fungi in wood. In 1878 Robert Hartig (*28, 29, 30*) showed that a certain fungus was responsible for a certain type of decay which had a fairly constant character in various species of wood. Later work in Germany was done by Falck (*22*) and Möller (*56*) on building rots, Czapek (*20*) on enzymic action, Münch (*57, 58, 59*), Mayr (*53*), Tubeuf (*79, p. 14–18, 31–44*), Neger (*60*) and others on biology of wood-rot fungi.

In England, Ward (*81, p. 287; 82; 83*), Biffen (*5, 6*), Bayliss (*4*) and Hiley (*35*) have contributed to the detailed information on wood rots and the fungi concerned. In Canada, Buller (*11, 12, 13, 14, 15*), Faull (*23, 24*), and White (*89*); and in the United States, Spaulding (*77, 78*), Schrenk (*68, 69, 70, 71, 72, 73*), Schrenk and Spaulding (*74*), Atkinson (*2*), Rankin (*61*), Long (*46, 47, 48, 49*), Meinecke (*54*), Weir (*84, 85, 86, 87*), and others (*1, 10, 33, 39, 41, 44, 65*) have given us valuable information on wood rots. The more recent contributions of Boyce (*7, 8, 9*) and of Kauffman and Kerber (*43*) deal mainly with incipient decay in wood. The papers in which cultural experiments with wood-rot fungi are presented have been comparatively few, that of Costantin and Matruchot (*17*) in France being one of the earliest. In some of the articles cited above, statements are found expressing belief that characters of decay may be used to identify the fungus causing the rot.

Since Hartig's time the presence of the fungus sporophore in close proximity to a specific decay in wood has been the single means of identifying that decay. However, the knowledge of wood rots has been gradually accumulating. The need for better diagnostic methods has become more and more apparent. Long and Harsch (*50*) have emphasized the importance of the cultural characters of fungi as diagnostic aids in establishing their identity. In the study here presented the author will attempt to show how gross, histological, and cultural characters may be applied to the diagnosis of decay in wood.

[1] Received for publication May 13, 1924—issued February, 1925. Paper in cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," pp. 365–567.

THE DIAGNOSIS OF DECAY

If a piece of wood which shows plainly that it is decayed has attached to it the fruiting body of a wood-rotting fungus, a probable diagnosis can be made by identifying the sporophore; but, if the sample bears no such fruiting body and shows little or no surface signs of decay, determining the causal organism is much more difficult. The writer's procedure is then as follows: First, the "unknown" is compared with a large number of "knowns." This has been made possible by the building up of a collection of some 600 or more typical rot specimens authenticated by the known identity of the sporophores collected on them. (Samples of wood rots collected from tree trunks which have lain on the ground for several years are not suitable on account of the possible presence of other wood-rot fungi in the same substratum.) These rots are grouped according to a classification presented below.

The next step is a study of the gross characters of the rot and comparisons with descriptive data on various rots. It is obvious that a collection and detailed descriptions of rots are essential in connection with studies of the gross characters. A key for the classification of wood rots will be the eventual outgrowth of these detailed descriptions.

Details of hyphae and cell structure in the infected wood are then studied under the microscope and the cultural characters of the organism, isolated from the infected wood and grown on artificial media, are observed. These cultures and the subcultures obtained from them are next compared with authentic stock cultures. At this point the identification of the organism can often be established with reasonable accuracy. A further test may be made to determine whether the fungus isolated from the unknown sample reproduces in sound sterilized wood an identical decay.

THE STAGES OF DECAY

Before the diagnosis can be discussed in detail, it is necessary to define the terms decay, decay stages, decay processes, and the classification of decays.

Decay (known to lumbermen as "dote," "doze," or "punky wood") is a process which begins with the development of wood-destroying fungi in wood and ends with more or less complete dissolution. The term includes all stages in the destruction process and two principal stages have been recognized by writers on wood decay. The early development of decay has been termed: Beginning, early, initial, incipient, advance, immature, first, primary, and invasion stage; and the final or decomposed stage: Final, last typical, advanced, mature, complete, ultimate, destruction. The two descriptive terms, invasion stage and "destruction stage," were extensively used by Falck (*22*). The terms "incipient" and "typical" have been selected by the writer to represent the two stages.

THE INCIPIENT STAGE

The incipient stage of decay corresponds to the period when the mycelium of the fungus is invading new host tissues preparatory to a more complete attack upon the cells (fig. 1, I). During this stage the hyphae ramify in all directions. In most decays the infected wood exhibits color changes although to the naked eye it appears to retain its structural characters, not having become punky, soft and spongy, stringy, ring-shaked or pitted. In some rots a softening of the wood may be detected in this stage and a characteristic brashness is evident, though with little or no discoloration.

Incipient discolorations and zone lines (*62*, *63*) usually accompany the incipient stage of decay. These are generally due to the presence of by-products resulting from the dissolution of the cell walls and cell contents by the fungous enzymes.

THE TYPICAL STAGE

The typical stage is that in which decay is plainly a factor in breaking down the wood cells. With few exceptions it is accompanied by unmistakable changes in strength properties of the infected wood, changes in color and changes in continuity and texture (fig. 1, T). One of the exceptions noted above is *Trametes pini* when the white pits are few and wide apart and the tissues are heavily infiltrated (*16*). Since infected wood undergoes color changes, the nature of the attacking fungus determines whether the rotted wood becomes whitish because of delignifying action, bleaching, or other causes, or whether the infected tissues become yellow, red, or brown due to a cellulose-dissolving action or to other causes not yet fully understood. The continuity of the wood tissues is broken in most decays in this stage. The wood

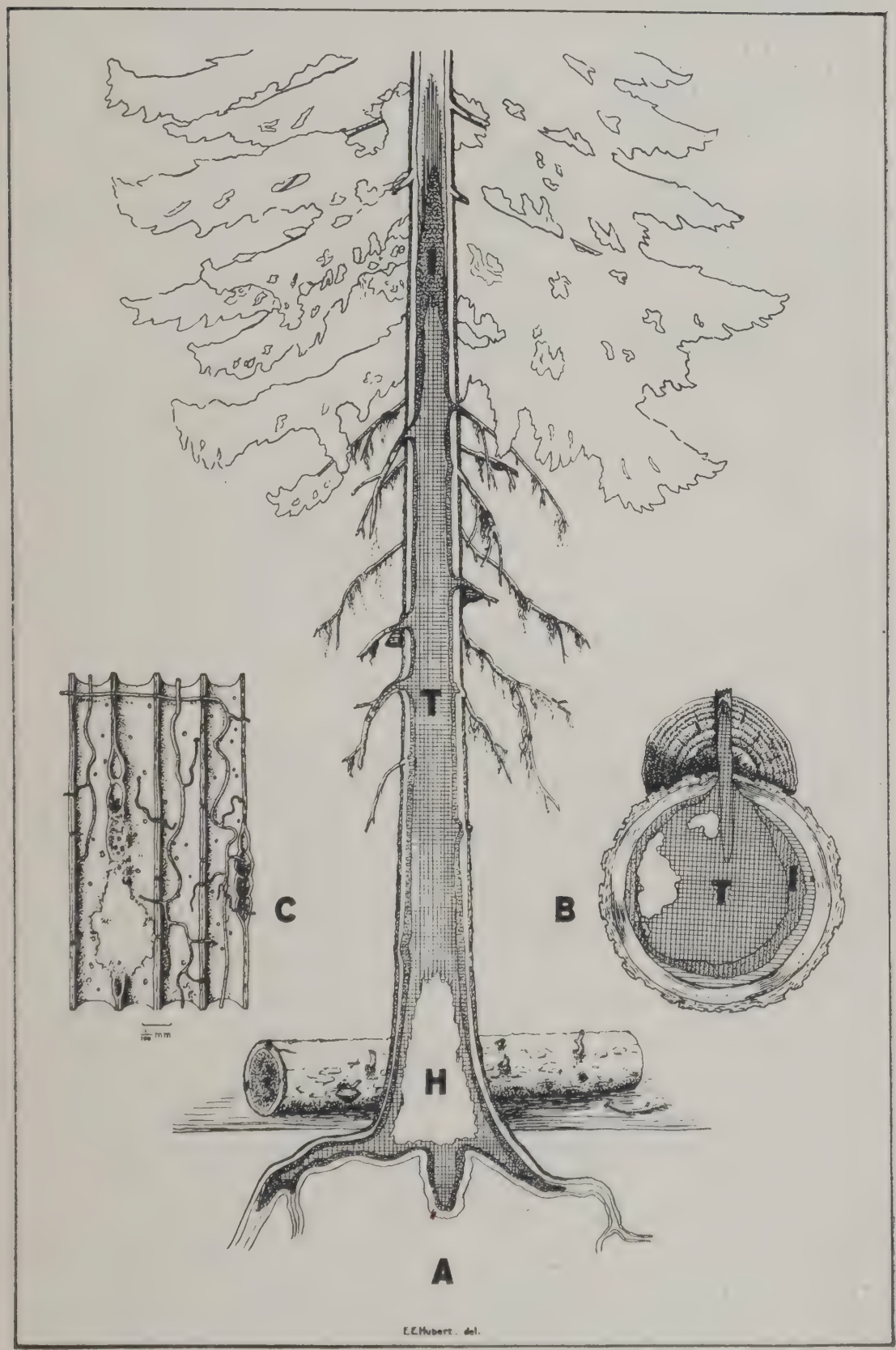


FIG. 1.—Heart rot in *Tsuga heterophylla* caused by *Echinodontium tinctorium* (Indian paint fungus). A.—Section through living tree showing the extent of decay with hollow cavity at H, typical rot at T, incipient rot at I, hidden phase of incipient decay beyond. The rot extends into the larger branches. B.—Cross section of the trunk showing relation of decay to sporophore and dead branch. C.—Tangential section of decayed wood showing the hyphae of the fungus penetrating the cell walls and decomposing them. Note the numerous bore holes.

may become soft and spongy and the remaining tissues become collapsed; and the result may be a stringy, spongy mass merging into hollow rot (fig. 1, A). Ring scale or ring rot may result from the more rapid dissolution of the cells in the spring wood of each annual ring, or a group of pits or pockets of isolated decayed tissues may be formed as in *Trametes pini*, *Stereum frustulosum* (Pers.) Fr., or *Polyporus amarus* Hedg. The texture of the infected wood varies from a soft, spongy mass to a shrunken, cracked mass of easily crushed material.

The terms *early incipient*, *incipient*, and *late incipient* may be used for the early stages, and *early typical*, *typical*, and *late typical* for the later ones, although hard and fast lines can not be drawn, as the process is continuous.

DECAY PROCESSES

Under certain conditions wood is very stable. Exclusion of air from it by immersion in water, or by thorough drying and subsequent exclusion of moisture, prevents decomposition by decay. However, all wood is subject to decay when attacked by the right organism developing under favorable conditions. Both heartwood and sapwood are attacked by wood-rotting fungi, the sapwood usually proving less resistant. Similarly, on account of its thinner cell walls and greater porosity or its different chemical composition, the spring wood of the annual ring offers less resistance to fungous attack than the summer wood. This often results in a type of decay called ring scale or ring rot, in which the annual rings split apart in a cylindrical plane.

The more pronounced decay processes result usually in either a white rot, or a brown rot, and this furnishes a general basis for classification. In some rots the two processes seem to be combined to a certain degree and a brown rot results, which contains areas resembling a white rot. Certain stages of the rot caused by *Echinodontium tinctorium* E. and E. show this characteristic.

Delignification apparently is the primary process in many of the white and brown rots. This results in the splitting by enzymic action of the so-called lignin-cellulose compound into a lignin complex (composed of hadromal, coniferin, and vanillin) and a cellulose complex (a polymer of starch). It rests with the particular attacking fungus whether the lignin complex is all or in part absorbed and the cellulose complex left (white rots) or whether the reverse action takes place (brown rots) (*11*, *20*, *28*, *63*). Some fungi are capable of absorbing both compounds in varying amounts, as will be shown later.

In some of the white rots the enzymic action continues and in the typical stage the lignin complex is often entirely removed, so that the presence of aldehydes can not be demonstrated with the use of indicators (*18*). In others a bleaching action takes place as the rot develops and the typical reaction wherein the lignin compound is removed seems lacking.

The cellulose complex is removed and apparently absorbed by the fungi producing brown rots. This cytolytic action usually leaves a brown, friable mass resembling charcoal in brittleness and composed mainly of the elements of the lignin complex. The decomposition may proceed by an attack upon the tertiary wall first and advance to the primary wall, or the process may be reversed. In these processes all stages from partial to complete disintegration of the cell walls are to be found. The bore holes produced by the hyphae in penetrating the cell walls are evidence of a complete localized destruction of the wall.

The dissolution of the primary cell wall may take place before or after the cytolytic reaction begins. Separation of the two halves of the primary wall in the incipient stage of decay is characteristic of certain rots. In the typical stage of other rots it may represent the last phase. An enzyme, pectase or pectinase, has been described as the ferment responsible for this action in various plant tissues (*20*, *42*). Czapek (*20*), discussing decay in wood, lists hadromase as the delignifying enzyme; cytase, the enzyme attacking cellulose; and pectase, the enzyme attacking the middle lamella of the cell wall. Schmitz (*66*) working with *Echinodontium tinctorium* E. and E. isolated a dozen different enzymes from the powdered mycelial mat developed in pure culture on carrot media. The same writer (*67*) working with *Polyporus volvatus* Pk. recorded 12 enzymes; and he listed 13 enzymes for *Fomes igniarius* (L.) Gillet. Zeller (*91*) determined the enzymes produced by *Lenzites sepiaria* growing on artificial media. A specific type of decay is thus produced in wood by a specific fungus; and a certain species of fungus produces quite similar decays in different species of woods, with occasional minor variations.

THE CLASSIFICATION OF WOOD ROTS

Very often decays are placed in two main divisions, heart rots and sap rots, on the basis of the parts of the wood attacked. The sap rots may be again divided into the decays of the live sapwood, such as those produced by *Polyporus dryadeus* or *Fomes igniarius*, and the decays of the dead sapwood and also heartwood, such as that produced by *Polystictus abietinus* Dicks. in conifers.

Frequently the heart rots are classified with respect to the longitudinal portion of the tree attacked. The decays found in the main portion of the tree are termed trunk rots (fig. 1, A); those found in the upper trunk, top rots; those found in the lower, basal portion, butt rots. The butt rots usually extend some distance into the heartwood of the roots (fig. 1, A). The decay produced by *Polyporus schweinitzii* Fr. is of this type. The term root rot is usually applied to a sap rot of live roots of which the *Armillaria mellea* (Vahl.) Quel. decay is a good example.

A classification based upon the particular reaction produced in the wood by the fungus seems of greater value in decay diagnosis. There are two groups due principally to the particular chemical or enzymic reaction of the fungus with the wood, which show the color distinction mentioned above (p. 526).

The white rots are caused mainly by the lignin-dissolving fungi, although they are not restricted to this type. They are usually whitish in the areas where the decay has reached the typical stage. These areas are principally composed of white cellulose compounds or are bleached to a whitish color as a result of the fungous action. The white rots may be divided into white pocket rots, white ring rots, white mottled rots, and white spongy rots (Pl. 1, A, B, C). The second group, comprising the brown rots, caused mainly by cellulose-dissolving fungi (but not limited to these), shows yellowish, reddish, or brownish discolorations in the typical stage. Usually the cellulose has been extracted for food by the fungus, leaving the lignin compounds in the remaining wood tissues. Under the brown rots of the coniferous woods the subgroups are brown pocket rots, brown ring rots and stringy rots, brown cubical rots, brown spongy rots, and brown mottled rots (Pl. 2, A, B, C).

Of the fungi treated in this study, *Trametes pini* (Brot.) Fr. (*Fomes pini* (Brot.) Lloyd and *Polyporus anceps* Pk., represent the white pocket rots. *Fomes igniarius* Linn. represents the white spongy rots; *Echinodontium tinctorium* E. and E. represents the brown ring rots and stringy rots; *Pholiota adiposa* Fr. represent the brown mottled rots; *Polyporus amarus* Hedg. represents the brown pocket rots; and *Polyporus schweinitzii* Fr., *Polyporus balsameus* Pk., *Lenzites sepiaria* Fr. *Trametes carnea* Nees. and *Lentinus lepideus* Fr. represent the brown cubical rots. The principal groups of wood rots are thus represented. More brown cubical rots than other types are discussed because this group appears to contain rots the incipient stages of which are often not distinguishable by discolorations of any kind, and which for this reason present a serious problem from the economic viewpoint.

Certain fungi produce in wood cells changes slightly resembling those found in true decay (*88*). These are the wood-staining or sap-stain fungi, of which the blue stain fungi, *Ceratostomella* spp., are representative (*34*). Their hyphae have been observed penetrating the tracheid walls in the woods of pine and other species (*36*, *37*, *57*, *72*). Penetration is not frequent, however, and is not so prominent a decomposition process as that produced in the medullary rays where the walls of the ray cells are often completely broken down. More commonly the hyphae in passing from cell to cell seek the natural openings, the bordered pits, where the netlike perforated membrane supporting the torus apparently presents an easy passageway, or they pass through the simple pits in the cell walls.

GROSS CHARACTERS OF DECAY

Color.—The discolorations usually associated with the stages of a particular decay are the most valuable diagnostic characters visible to the naked eye (Pls. 1 and 2). They vary greatly for different fungi and slight variations may be noted for the same fungus in different hosts. Color forms the basic part of rot descriptions, but these descriptions, of course, can not be presented in detail here. However, the discolorations produced in wood by the advancing hyphae of wood-rotting fungi should be given more than passing attention because it is the incipient stage that in many cases eludes detection.

Only a few important rots have striking or even visible incipient discolorations. These are more common in

white rots, but quite absent in brown rots, particularly brown cubical rots and brown pocket rots.

The incipient discoloration produced in wood by *Trametes pini* (Pl. 3, A) exemplifies the invasion discoloration found in many white pocket rots. It is so common in the lumber and timber of commerce that it is known as "red heart" or "firm red heart." It occurs in nearly all species of coniferous wood and is almost entirely confined to the heartwood. According to the host and the conditions of development of the fungus, the "red heart" varies from pinkish to dark reddish or brownish or sometimes a purplish color. While the appearance of a distinct color in the wood may indicate the presence of decay, identification is complicated by the fact that such fungi as *Polyporus anceps* Pk. and *Fomes annosus* Fr., which produce white pocket rots, also cause pinkish to reddish discolorations in the incipient stage of rot. *Polyporus circinatus* has recently been collected in Minnesota on roots of living *Picea mariana*, producing in this wood a white pocket rot having a light to dark reddish incipient discoloration. This observation, verified by cultures, adds another to the list of fungi producing reddish discoloration in heartwood.

The incipient stages of certain brown rots, such as produced by *Fomes laricis*, *Polyporus sulphureus*, *Polyporus schweinitzii*, *Fomes pinicola*, and others, in certain hosts may often be confused with each other or with the invasion discolorations produced by the white pocket-rot fungi. The natural pinkish or reddish color of the heartwood of certain hosts, such as *Pseudotsuga taxifolia*, adds to the confusion. Certain invasion discolorations take on the nature of a water-soak in the infected wood of freshly cut trees. This is characteristic of the incipient stages of the white rots produced by such fungi as *Ganoderma tsugae* in *Tsuga canadensis* (Pl. 3, B), and *Polyporus dryophilus* in broadleaf hosts. According to Long (*46*, *48*), *Polyporus pilotae* and *Stereum subpileatum* also produce a water-soak in the incipient stage of decay. In the case of *Ganoderma tsugae* the water-soaked areas soon lose their definition on drying, and little or no discoloration remains to indicate the incipient decay areas.

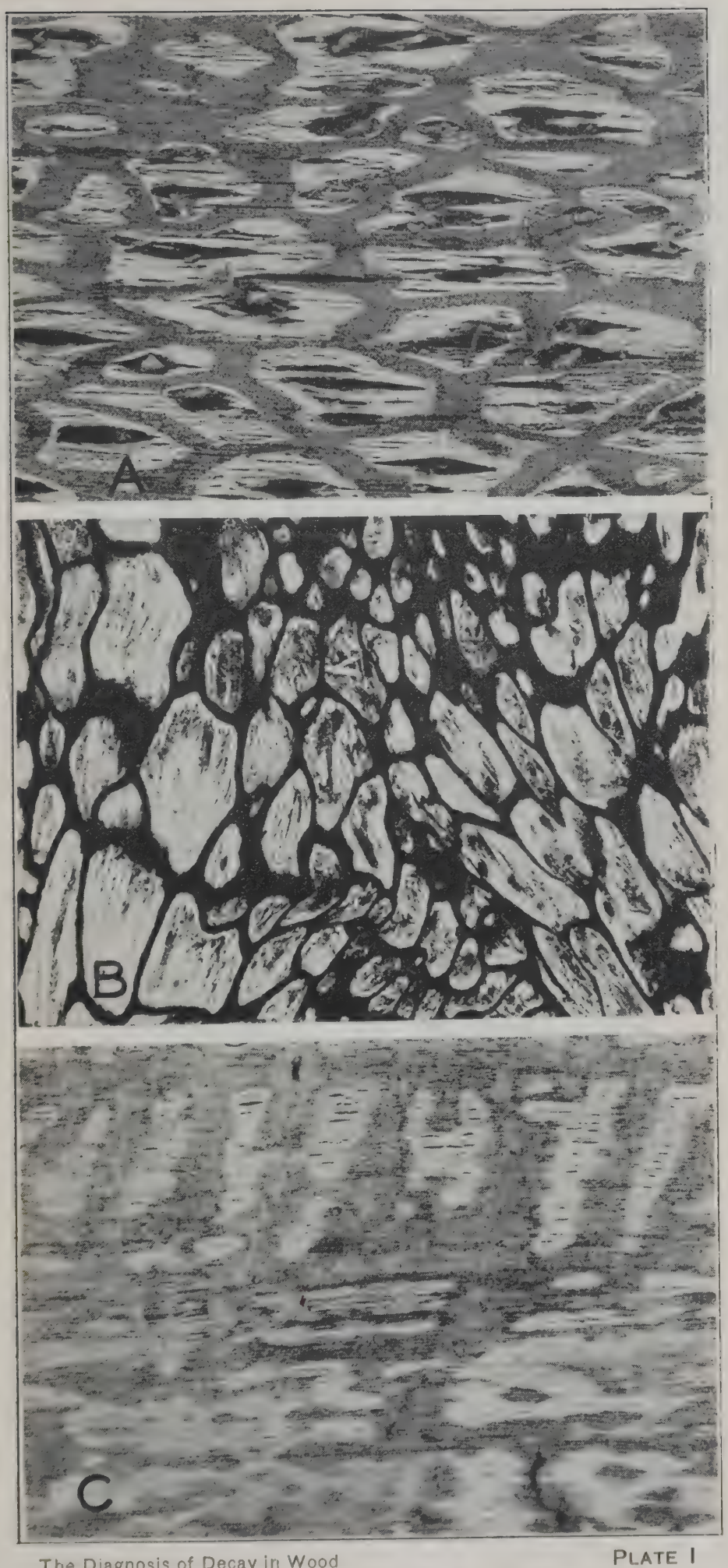
In the incipient stage of decay produced by *Echinodontium tinctorium* the incipient discolorations extend some distance ahead of the typical stage (fig. 1 and fig. 2, B). Where the discolorations fade out and for some distance beyond this limit, the action of the fungus on the wood structure continues. Consequently, sound-appearing boards cut from infected areas in the logs show a brashy, crumbly texture which unfits the stock for commercial use when seasoned (*9*, *54*, *85*). *Polyporus anceps*, probably identical with *Polyporus ellisianus* as used by Long (*49*), produces in *Pinus ponderosa* a characteristic incipient discoloration (fig. 2, A), which so far has not been observed in other hosts of the fungus. Reddish to reddish-brown areas radiate from typical rot areas viewed in transverse section. Lighter reddish discolorations have been noted in *Picea canadensis* infected with *Polyporus anceps*. White (*89*) states that when *Fomes applanatus* develops in living hosts the incipient decay is bordered by a brownish invasion zone one-fourth inch wide or wider.

White considers this character is of diagnostic value. *Fomes igniarius* produces in the incipient area a characteristic invasion zone, usually a dark band or zone of irregular outline and width (fig. 2, C). *Pholiota adiposa* produces in *Tilia americana* a dark brown, characteristic invasion zone which is often included within the area of typical decay as the invasion progresses (fig. 2, D). Pronounced incipient discolorations are not observed frequently in the brown rots. The rot caused by *Fomes pinicola* in *Tsuga heterophylla* is an example, showing but a faint discolored area of incipient decay which does not form a continuous band around the typical rot areas (fig. 2, E). Some incipient and typical discolorations common to commercial woods are well illustrated in the work of Boyce (*9*).

The difference between white and brown rots in the typical stage is usually very clear. White rots commonly show distinctive incipient discolorations and brown rots do not. Hence brown rots probably cause greater economic loss. The difficulty in diagnosis often lies in distinguishing decays in which the incipient discol-

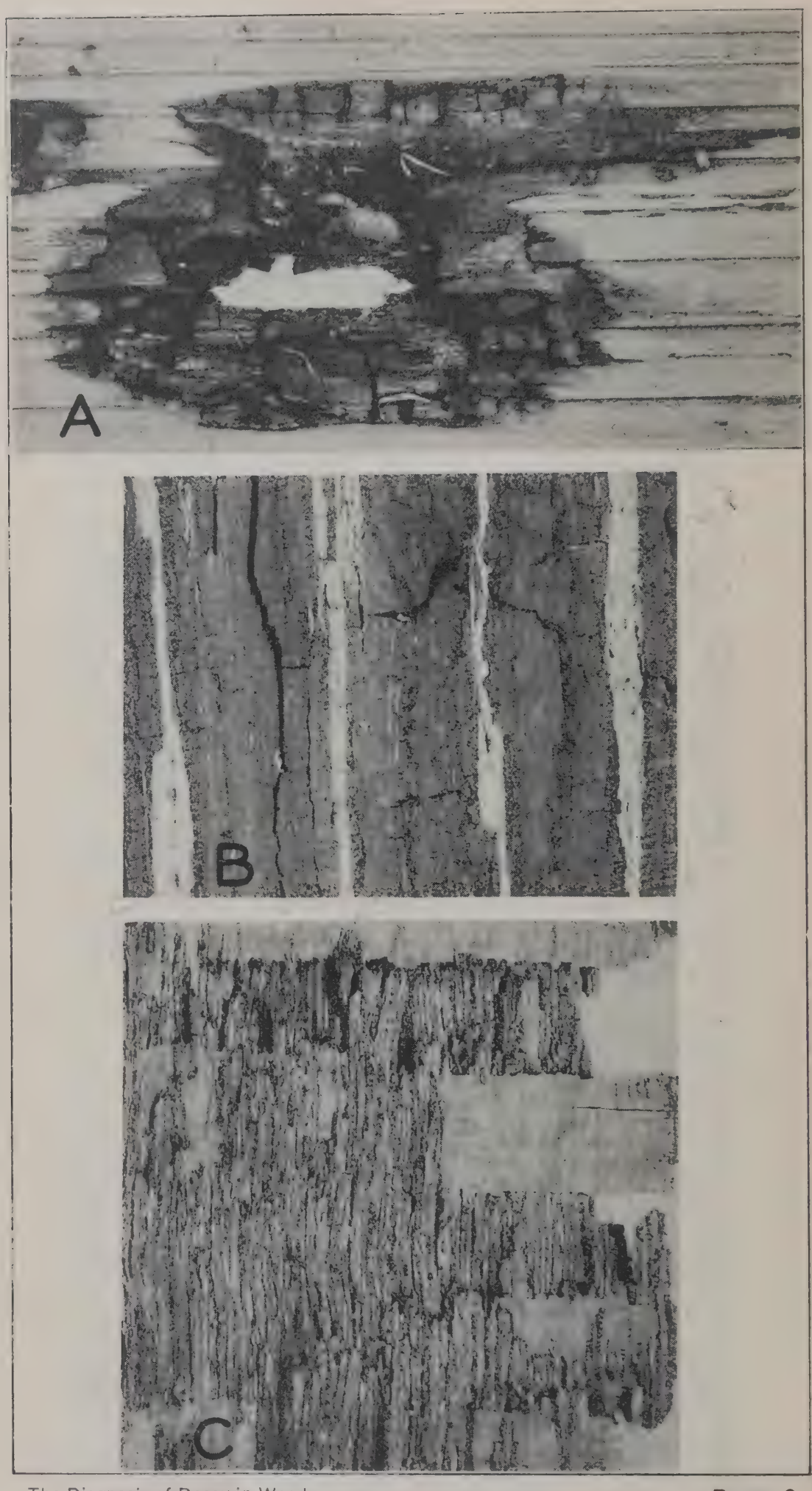
EXPLANATORY LEGEND FOR PLATE 1

Three typical white rots (about natural size). A.—White pocket rot caused by *Fomes nigrolimitatus* (*F. putearius* Weir) in *Larix occidentalis*. Tangential section. B.—White pocket rot caused by *Fomes extensus* in a hardwood root (tangential section). C.—White mottled rot caused by *Fomes applanatus* in *Populus grandidentata*. Radial section. Dark-colored zone lines often accompany this rot.



The Diagnosis of Decay in Wood PLATE I

(For explanatory legend see p. 528)



The Diagnosis of Decay in Wood

PLATE 2

(For explanatory legend see p. 531)

orations as well as the typical characters resemble each other closely.

ZONE LINES.—Thin zones of discolored matter usually appearing in cross section as narrow to broad lines, particularly those associated with incipient decay, are valuable diagnostic characters (Pl. 4), though additional evidence is usually necessary. They are usually formed in the white rots only, being rare in brown rots. Their cause is still unsettled. Rhoads (*62*) concludes that the brown substance (decomposition products) commonly occurring in dicotyledonous woods attacked by wood-destroying fungi arises only after the death of the cells through the oxidation of their contents and certain constituents of their walls, occurring most notably in the parenchyma cells.

Observations on zone lines formed between two fungi occupying the same wood tissues indicate that this type of zone line is fairly common. But in several cases these zone lines differed from the clear-cut, brightly colored, narrow lines formed through desiccation, which will be discussed later. The zone lines between the rots produced by *Polyporus anceps* and *Lenzites sepiaria* in *Picea canadensis*, for example, are broader, up to one-fortieth inch wide, with indistinct edges and of a dull brownish color. Very often a double zone line is formed. The zone lines between rots of *Fomes applanatus* and *Stereum frustulosum* in *Quercus* sp. resemble the above closely, but are fainter and less distinct. The zone lines of *Fomes applanatus* are usually narrow, brownish black to black, sharply defined, and rarely doubled.

Schrenk (*73*) mentions the orange-colored zone lines accompanying the decay produced by *Polyporus adustus* in the sapwood of red gum (*Liquidambar styraciflua*). The zone lines produced in wood inoculated with pure cultures of a fungus (Table I) isolated from sap-gum boards showing a white rot with orange zone lines, and produced in pure cultures on malt agar are identical in width and color with those found in infected sap-gum lumber. However, the typical rot produced in this experiment is a white spongy rot and in the writer's experience the typical rot of *Polyporus adustus* in certain broadleaf hosts is always a white mottled rot and is very characteristic. Whenever the white mottled rot was observed in connection with sporophores of *Polyporus adustus*, the orange zone lines were lacking. This would indicate that some fungus other than *Polyporus adustus* may be responsible for the white spongy rot and orange zone lines in sap gum.

Some writers (*84*, *89*) attribute the zone lines in infected wood to reactions between two wood-inhabiting fungi occupying the same substratum. White (*89*) in his description of the rot produced by *Fomes applanatus* states that—

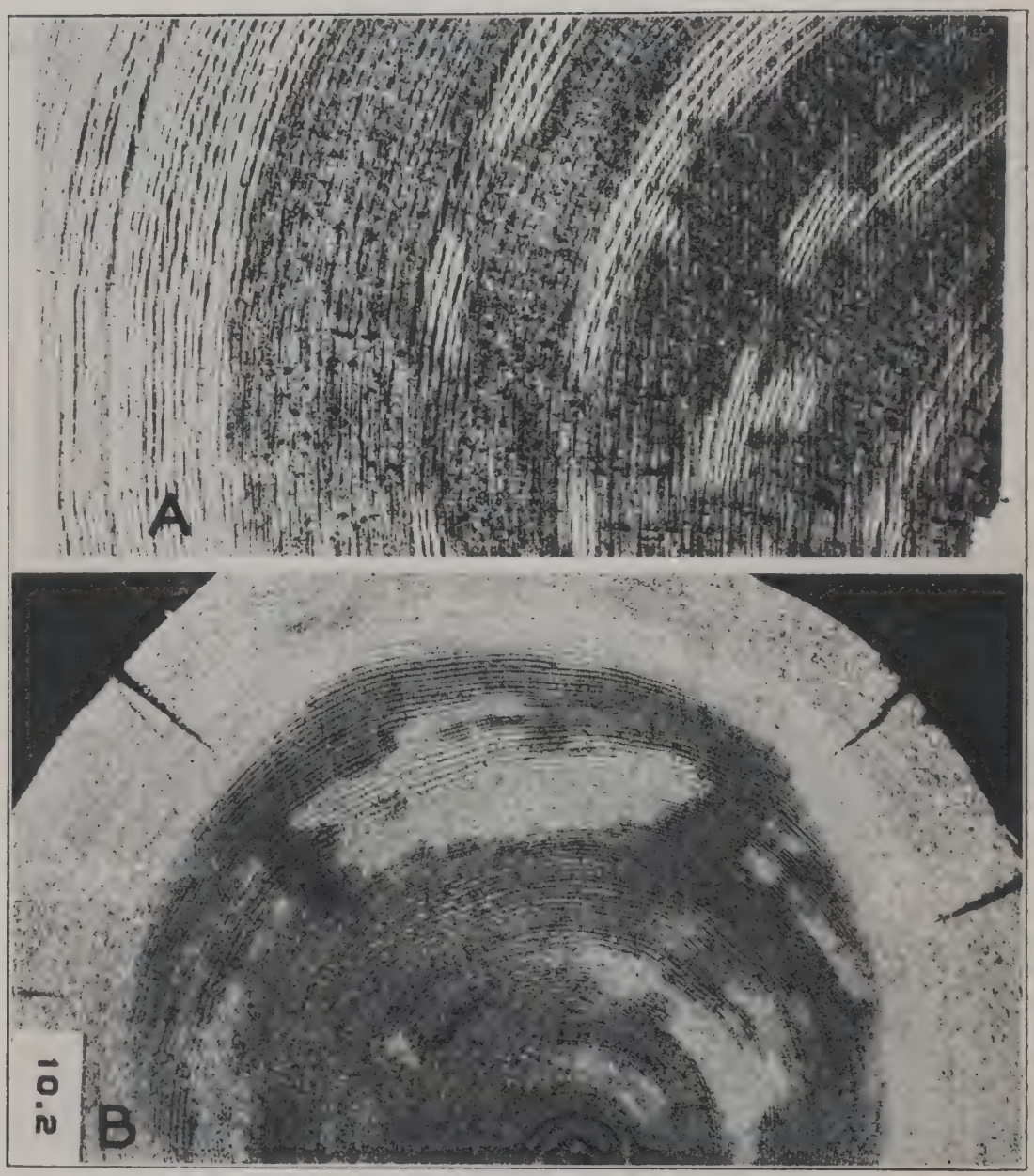
> black lines are encountered but cultural or other evidence shows that when they do occur more than one species of fungus is at work. They are produced at the point of contact of two invading fungi. * * *

If this proves generally true, another type of zone line undoubtedly not caused by contact of two invading fungi must be accounted for. A study of the characteristics of a large number of wood rots shows that many of them have zone lines included within the typical rot areas (Pl. 4). Although such areas, including zone lines, were cultured repeatedly, no fungus other than the one responsible for the decay developed in any case. Field observations indicate that these zone lines occur more commonly in the pieces of infected wood most subject to desiccation. Hartig (*28*, *p. 32–39*) states that the zone lines in oak wood infected with *Fomes igniarius* (*Pl. 15*, *fig. 2*) are due primarily to the entrance of air into the infected wood tissues. Lindroth (*45*) figures a zone line formation just back of the freshly-cut surfaces of birch wood infected with *Polyporus nigricans*.

Additional observations, given in Table I, were made on large pieces of infected wood brought in fresh from the woods. The pieces when cut at the time of collecting were in most cases completely infected but showed no zone lines; when cut open after a period of drying in the warm air of the laboratory, many of the rots showed typical zone lines extending transversely across and but a short distance back from the ends of the infected log sections. Desiccation and oxidation of the decomposition by-products seem responsible for the formation of these lines. Data

EXPLANATORY LEGEND FOR PLATE 2

Three typical brown rots (about natural size). A.—Brown pocket rot caused by *Polyporus amarus* in *Libocedrus decurrens*. "Pecky" incense cedar (radial section). B.—Brown cubical rot caused by *Fomes pinicola* in *Tsuga canadensis*. Note the white mycelial mats in the cracks. Radial section. C.—Brown spongy rot caused by *Polyporus berkeleyi* in *Quercus alba*. "String and ray rot." The rays are left intact and form a remarkable contrast to the crumbly, spongy condition of the adjoining tissue. Radial section.



The Diagnosis of Decay in Wood

PLATE 3

A.—Transverse section of southern yellow pine wood infected with *Trametes pini* showing the characteristic invasion or incipient rot area preceding the typical rot containing pockets (about natural size).

B.—Transverse section of a log of *Tsuga canadensis* infected with *Ganoderma tsugae* showing the dark "water soak" appearance of the incipient stage of the rot. Photographed without color screen, soon after cutting (about ⅕ natural size).

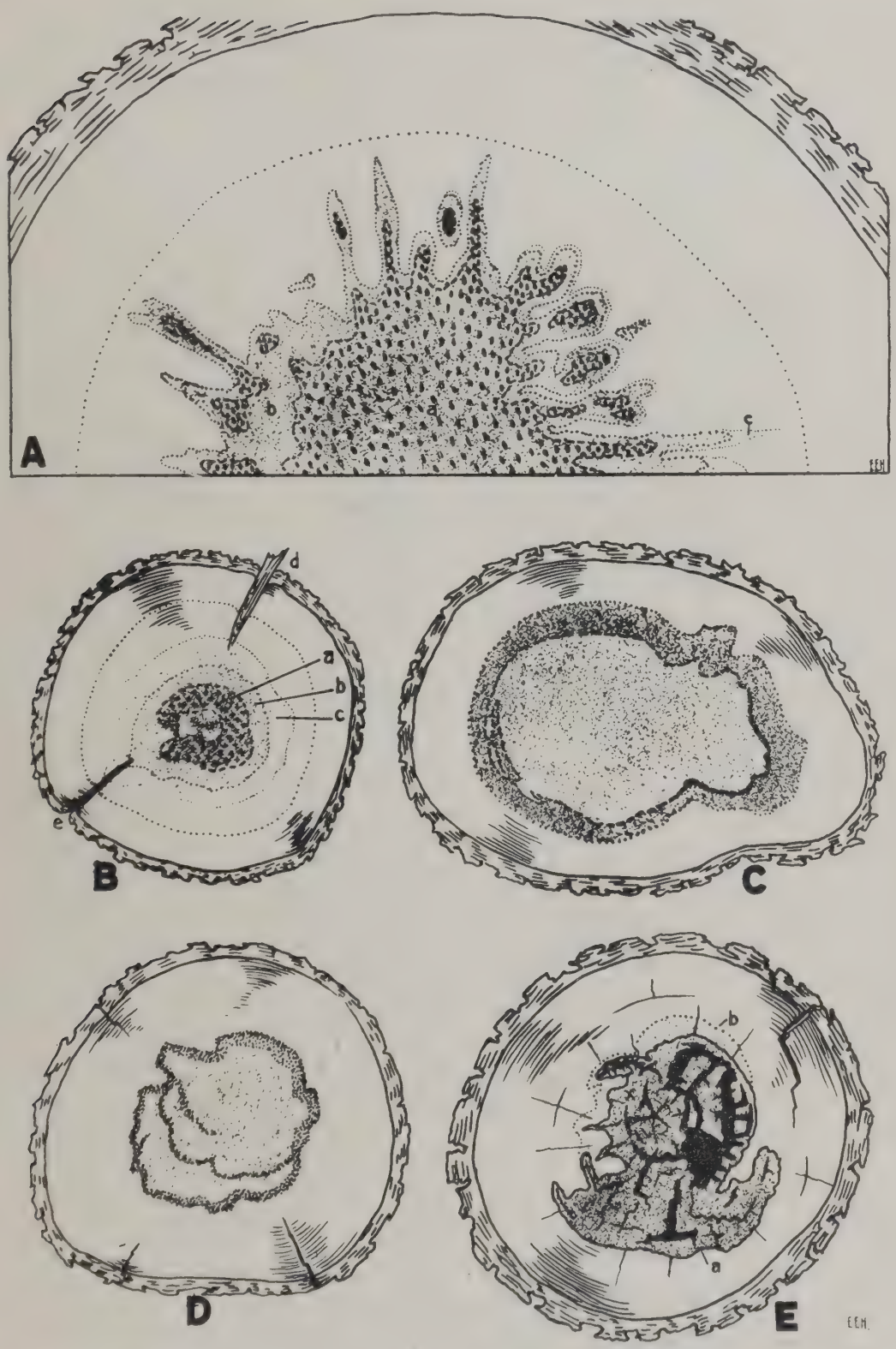


FIG. 2.—A.—Transverse section of *Pinus ponderosa* infected with *P. anceps* (*P. ellisianus*). Typical grayish-white pocket rot at *a* and similarly shaded isolated areas. Red-brown incipient rot at *b*. At *c* a faint yellow-brown invasion zone which appears only in part of the infected wood. Note the radiate development of the rot. One-fifth natural size. B.—Transverse section of *Tsuga heterophylla* infected with *Echinodontium tinctorium*. Typical brown stringy rot at *a*, typical rot not stringy in texture at *b*, typical rot grading into incipient rot at *c*. A very faint discoloration extending from incipient area at edge of *c* up to sapwood zone at dotted line. Dead branch stub at *d* and unhealed frost crack at *e*. One-sixteenth natural size. C.—Transverse section of *Acer rubrum* infected with *Fomes igniarius*. Typical rot in central shaded area surrounded by the darker band of the invasion zone. One-sixteenth natural size. D.—Transverse section of *Tilia americana* infected with *Pholiota adiposa*. Typical rot in central shaded area surrounded by the darker band of the invasion zone. Note the earlier invasion zones still showing and included within the typical rot area. One-sixteenth natural size. E.—Transverse section of *Tsuga canadensis* infected with *Fomes pinicola*. Typical brown cubical rot at *a*. Faint yellowish-brown discolored areas of incipient rot at *b*. One-sixteenth natural size.

TABLE I.—*Formation of zone lines in various species of infected wood after drying*

Fungus	Host	Date collected	Condition	Date examined	Zone lines across ends of sample
Fomes applanatus	*Acer saccharum*	July 1921	Fresh; mine timber; no zone lines.	Nov. 3, 1921	Narrow, continuous, dark brown, ⅛ to ¼ inch back from surfaces.
Do	*Malus* sp	1918	Old specimen; no zone lines.	Aug. 16, 1921	Narrow, brownish black parallel to cut surfaces, 1/16 to ⅛ inch back from surfaces.
Do	*Hicoria ovata*		Fresh; no zone lines	Mar. 8, 1922	Narrow, black, 1/16 inch back from one end of sample.
Do	*Acer negundo*	Nov. 26, 1922	do	Dec. 7, 1922	Narrow, continuous, black to brownish, ⅛ to ¼ inch back from surfaces.
Fomes everhartii	*Quercus* sp	May 18, 1921	No zone lines	June 10, 1921	Narrow, black, irregular, not parallel, ½ to 1 inch back from surface.
Fomes fomentarius	*Betula occidentalis*	Sept. 1921	Fresh; no zone lines	Dec. 2, 1921	Narrow, brownish black, 1/32 inch back from surfaces.
Fomes igniarius	*Ulmus americana* logs	Sept. 12, 1921	do	Nov. 17, 1922	Narrow, continuous black, ½ inch back from ends of logs.
Echinodontium tinctorium	*Abies grandis*	Apr. 24, 1923	Fresh; zone lines in typical rot areas.	May 12, 1923	Reddish, irregular lines along edges of loose fibrous areas of rot.
Ganoderma tsugae	*Tsuga canadensis*	Sept. 17, 1921	Fresh; no zone lines	Oct. 12, 1921	Narrow, continuous black, ¼ to ½ inch back from one end surface.
Polyporus betulinus	*Betula papyrifera*	Sept. 17, 1921	do	Nov. 11, 1921	Zone lines observed across ends of sample.
Schizophyllum commune and *Polystictus pargamenus.*	*Populus grandidentata* branch	June 21, 1921	Fresh; zone lines parallel to branch.	June 27, 1921	Narrow, continuous, brownish black, almost parallel to surfaces, ¼ to ½ inch back from surfaces of both ends.
Stereum sulcatum	*Larix occidentalis*	Apr. 24, 1923	No zone lines	May 15, 1923	Narrow, black, roughly parallel to ends, ¼ inch back from surfaces.
Trametes pini	*Picea canadensis*	June 1921	Few lines in typical rot areas.	Nov. 23, 1921	Narrow, dark brown to black, ⅛ to ¼ inch back from end surfaces.
Do	*Pinus strobus*	Sept. 1921	No zone lines	July 8, 1922	Narrow, dark brown, ⅛ inch back from end surfaces.
Do	*Picea sitchensis*		Inoculated with pure culture; the inoculated block dried out.	Nov. 23, 1921	Narrow, continuous, across both ends of blocks.

on the appearance of zone lines in wood inoculated with pure cultures of wood-destroying fungi support this view. The zone lines invariably appeared during the periods when the moisture in the tubes and in the inoculated blocks had nearly evaporated. Zone lines were secured in the following cultures on the hosts named: *Trametes pini* in *Picea sitchensis* heartwood, *Fomes igniarius* in *Populus tremuloides* sapwood, *Xylaria polymorpha* in *Tilia americana* heartwood, *Polyporus adustus* (?) in *Populus tremuloides* sapwood, *Hymenochaete rubiginosa* in *Populus tremuloides* heartwood, *Fomes applanatus* in *Populus tremuloides* sapwood, *Ganoderma curtisii* in *Populus tremuloides* sapwood and in pure cultures on malt agar, the mycelium extending to the cotton plug and forming a zone line across the plug.

The data in Table I indicate that whenever freshly cut pieces of wood infected with certain fungi are placed in a dry room or left to dry in the open, characteristic zone lines are formed a short distance back and parallel to the cut surfaces (Pl. 4, A, B, C). Field observations often show zone lines in the upper portion of rotted stumps in the region where excessive drying occurred, roughly parallel to the surfaces from which the evaporation took place.

Zone lines may be regarded as additional evidence or as aids in diagnosing decay, but in most cases can not be regarded as infallible characters in determining the causal organism. The data indicate that one type of zone line is commonly formed during desiccation. Other types, less common, have various origins. The entire problem of zone lines needs to be attacked from the microchemical angle in order to determine the fundamental causes for these characteristic despoits. In this way their diagnostic value may be more fully developed.

Texture.—Texture may be determined by hardness, elasticity, brashness, or ease of crushing or crumbling. Hardness may be tested in a number of ways, but the comparative resistance offered when a knife blade or a dull point is jabbed into sound and decayed wood has been found fairly satisfactory. Prying up slivers of the wood with a knife blade often furnishes comparative evidence. This common test for elasticity in general use by inspectors (*7*) is of value since the sounder wood pulls out with a more splintery appearance and shows greater elasticity and coherence than the infected wood. The manner in which fibers tear out at the saw cuts is also a reliable sign of decay.

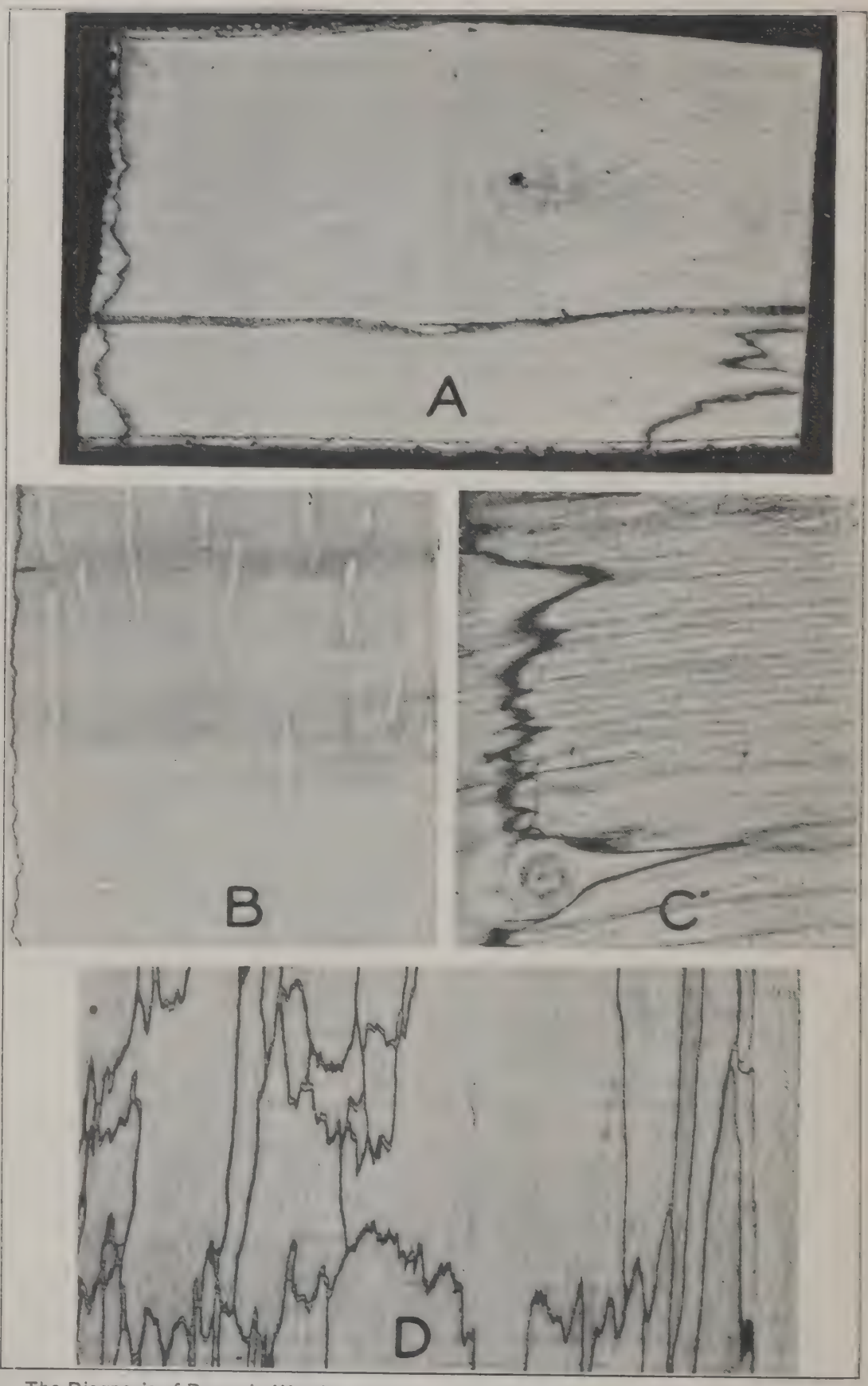
Brashness.—The tendency to break without a splintering fracture is often an indication of decay. Out of several thousand specimens of Sitka spruce tested at the Forest Products Laboratory for their mechanical strength properties, 16 were selected as representing the splintering or tough type of failure and 15 as representing the brash or weak type. The section of wood technology of the Forest Products Laboratory made a careful study of the wood structures of each piece and found no definite physical cause for brashness. Later the writer found minute hyphae of a wood-destroying fungus in nearly all of the 31 brash sticks (Pl. 5). Only one of them showed faint rot discolorations, the remainder appearing normal. Two of the tough sticks contained blue-stained sapwood and the third contained only a very few hyphae of the wood-destroying type of fungus. Brashness may, of course, be due to other factors also.

Odor.—*Polyporus schweinitzii* rot in Sitka Spruce has a noticeable anise-oil odor, and *Polyporus berkeleyi* rot in oak also has a peculiar odor of anise oil. A turpentine odor accompanies the typical stage in most conifers. A somewhat similar odor accompanies the rots caused by *Lentinus lepideus* Fr. and by *Polyporus fissilis*.

Chromo - chemical tests. — These are the gross color reactions produced by the application of chemicals to the surface of the decayed wood. These chemicals are principally so-called lignin or cellulose indicators, and according to Crocker (*18*) the aldehydes usually present with lignin compounds are responsible for the specific color reactions.

MICROSCOPICAL CHARACTERS OF DECAY

The hyphae present in infected wood are usually of two kinds, the older, of large size, sometimes colored and usually much branched or anastomosed; the younger, very small, hyaline, and irregularly branched. Often these young hyaline hyphae are very difficult to detect even with the aid of suitable stains and oil immersion lenses (Pl. 5). With the exception of the hyphae of some of the sap-stain fungi, the hyphæ found boring through the cell walls are rot producers. Hyphae may be found in the medullary rays, in tracheids, in vessels, in sapwood or in heartwood, and may be colored or hyaline. Clamp connections sometimes called buckles or nodose septae usually indicate a wood de-



The Diagnosis of Decay in Wood

PLATE 4

A.—Zone lines in living *Betula papyrifera* infected with *Fomes fomentarius*. B.—Zone line developed in drying *Acer saccharum* infected with *Fomes applanatus*. Note the direction of the line paralleling the freshly cut end. C.—Zone line in *Picea canadensis* infected with *Trametes pini*. The line follows irregularly behind the cut and extends inward where a knot intervenes. D.—Zone lines produced in fallen trunk of *Acer rubrum* by *Xylaria polymorpha*. Zone lines are formed around the decayed areas which originated from various foci of infection, producing double lines. About ⅘ natural size.

Photomicrograph showing minute hyaline hyphae of a wood-destroying fungus (*P. schweinitzii*) in a test piece of *Picea sitchensis*. Note the penetration of the cell wall as indicated by the darkened circular areas in the path of the hyphae ×1300. Stained with Bismark brown and methyl violet.

stroyer. So-called medallion hyphae are common to *Lenzites* spp. (*22*). Granular deposits on the outer walls of the hyphae are characteristic of certain species of Peniophora or Coniophora and sometimes of *Lentinus lepideus* (*11*, *22*).

The order of dissolution of the cell wall may often suggest the type of decay. If the primary wall is resistant the bore holes may be constricted (19 in fig. 6). Spiral cracks in primary and secondary walls and bore holes or cell-wall punctures are often reliable characters; also spiral shrinkage cracks extending from pit openings or bore holes (fig. 5, E, F). The latter have also been found in wood in which hyphae were absent. If it is proved that these cracks are due to fungous action, their presence in tissues lacking hyphae of any kind may point to enzymic action some distance removed from the secreting hyphae. Corrosion marks, grooves, uneven surfaces, and uneven thicknesses of cell walls, splitting of middle lamella, and dissolution of tertiary layer are all good indications of decay. White (*89*) considers overabundant formation of tyloses in the region of incipient decay, and none in the sound region beyond, a safe criterion of the presence of a parasitic wood-rot fungus. Wound gum deposits in the region ahead of hyphae are often characteristic signs. Calcium oxalate crystals deposited on the cell walls and in form resembling hyphae may indicate (*Lentinus lepideus*) hyphae which have been decomposed (*11*). Other reliable signs of decay are the enlargement of pit openings, corrosion and cracking of bordered pits, and presence of granular deposits or corrosion marks on the scalariform bars at ends of vessels. The accumulation in the bordered pits of by-products and resins in certain woods should not be confused with corrosion marks often seen on the embossed portion of these pits.

Corrosion, thinning, and entire decomposition of the cross walls are prominent decay characters in the medullary rays. Whether pit openings or bore holes are used as channels for hyphae may aid in distinguishing wood destroyers from staining or molding fungi. In the ray cells, decomposition products, wound gum, granular deposits, and tannin are often of diagnostic significance. Where the enzyme amylase is produced the starch grains are corroded or are partly or completely dissolved. The effect upon the wood-fiber cell walls of a decay-producing fungus is well illustrated in Plate 6. A tranverse section of the wood of *Ulmus americana* infected with a white rot fungus in which the fiber walls are remarkably reduced is shown at *A*, while *B* shows a transverse section of the normal uninfected wood of *Ulmus americana* with thick fiber walls.

Color reactions produced in microscopical sections by different chemicals as indicators of the presence or absence of lignin or cellulose compounds in the infected tissues are useful in decay diagnosis and in a study of the microscopical characters of particular wood rots. Many such chemicals are given by Crocker (*18*). The writer has found the following of value also: Para-nitroaniline and hydrochloric acid, producing a bright orange yellow; pyrrole, producing a deep red; diphenylamine, producing an orange yellow; and both metol and hydroquinone, giving a light yellow color reaction.

For testing the presence of cellulose the iodine compounds giving violet to blue reactions are often helpful, also sulphuric acid and cuprammonia. A test for tannin is sometimes of value, as tannin is often found in decayed wood and absent in sound wood of the same species. Tubeuf (*79*, *p. 40*), using the ferric chloride test, found no reaction in sound spruce wood but secured a reaction for tannin in the same wood infected with dry rot produced by *Merulius lachrymans*. The standard stains used in microscopical technique serve to determine the constituents or the layers of the cell wall first attacked and whether dissolution is from within the cell outward or the reverse. The action of the fungus on the middle lamella as determined by staining is of particular value, as shown by the work of Spaulding (*77*).

It has been extremely difficult in the microscopical examination to demonstrate the presence of fungous hypae in the wood tissues. They are very small and quite transparent in the incipient stages of many rots, and are easily confused with hyaline fragments of the wood cells. The methyl violet Bismarck brown method for staining

EXPLANATORY LEGEND FOR PLATE 6

A.—Photomicrograph showing transverse section of *Ulmus americana* infected with a wood-destroying fungus. Note the thinness of the wood fiber cell walls. ×50. B.—Photomicrograph showing transverse section of sound *Ulmus americana*. The thickness of the normal wood fiber cell walls are very pronounced. ×50. (Courtesy of Mr. Koehler.)

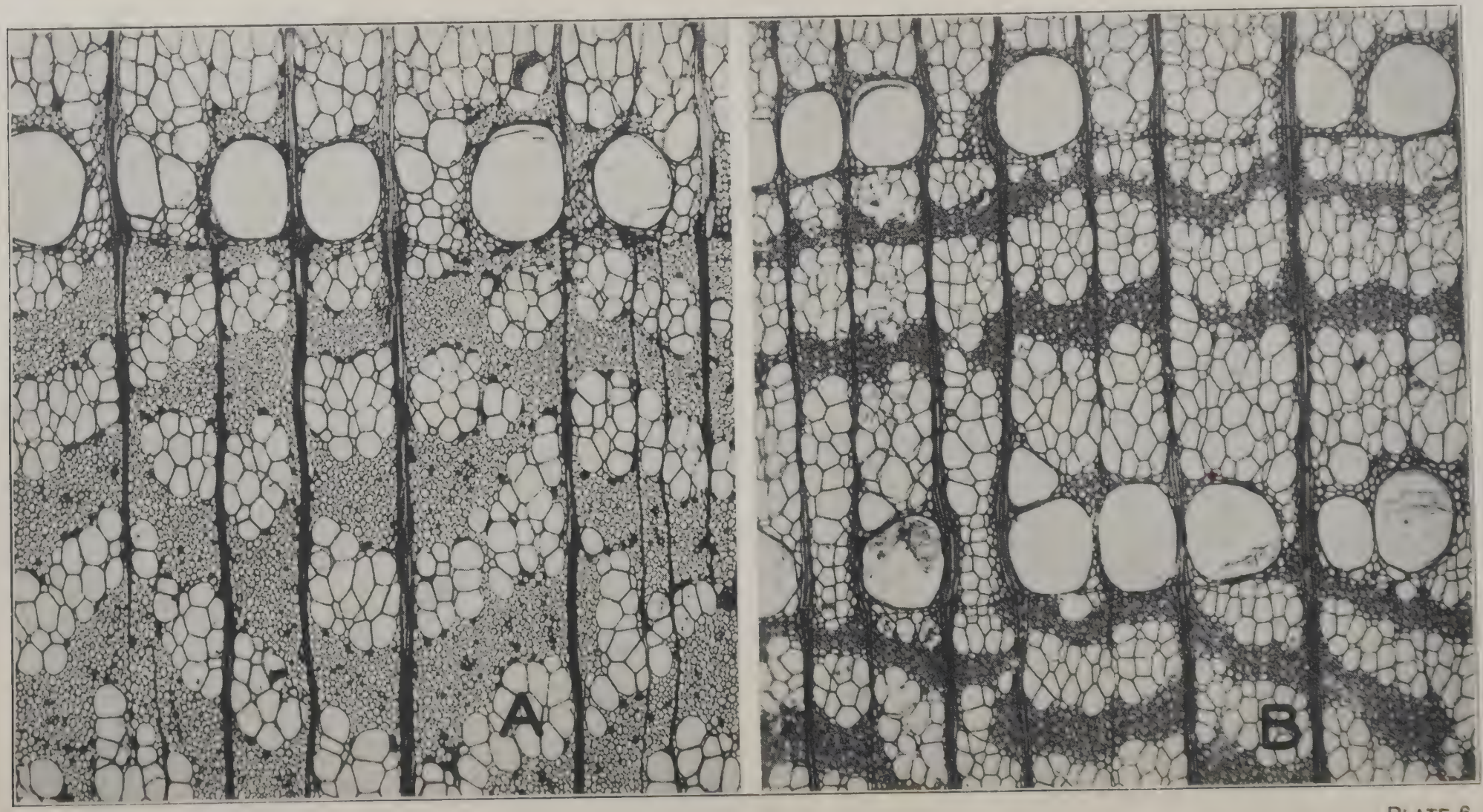


PLATE 6

(For explanatory legend see p. 538)

hyphae in infected wood has been used with some success in commercial work (*38*). Other useful stains (requiring longer preparation of the slides) have been developed by Sinnott and Bailey (*75*) and by Diemer and Gerry (*21*).

EXTENT OF HYPHAE IN AND BEYOND DISCOLORED AREAS

There seems to be very little histological and no cultural evidence in literature that the hyphae of certain wood-inhabiting fungi extend in and beyond the invasion discolorations.

Meinecke (*54, p. 28*) states that the incipient stage of rot due to *Echinodontium tinctorium* has a sound appearance but "has only recently been invaded by the mycelium." He terms the invading hyphae "pioneer hyphae."

Boyce (*8*) in describing the hyphae of *Polyporus amarus* in infected incense cedar (*Libocedrus decurrens*) states that—

> hyphae were commonly present in the apparently sound wood surrounding young pockets to a distance of 4 mm. (0.157 inch) and sparingly from that point to 8 mm. (0.314 inch) in a horizontal direction." In the case of the last (highest) pocket in a diseased tree the hyphae were abundant to a distance of 1.5 cm. (0.6 inch above the pocket) and sparingly from that point on to 7.8 cm. (3.07 inches), where they ended

Hiley (*35, p. 88*) in discussing the incipient rot produced by *Fomes annosus* in larch wood states that "stray hyphae were often found in the red rot region, and where hyphae could not be seen, the occurrence of the empty bore holes proved that they had formerly been present. Hyphae were even seen in the normal colored wood outside the turpentine region (bordering incipient decay and infiltrated with resin).

White (*89, p. 156*) states that the brown invasion zone noted in living trees attacked by *Fomes applanatus* "keeps pace with the advancing hyphae." This would imply hyphae present in this zone.

Schrenk (*68*) describing the mycelium of *Polyporus juniperinus* in red cedar (*Juniperus barbadensis*) states that

> the mycelium of the fungus is found in the wood between the holes (rot pockets), as well as in the sound (?) wood around the cavities,

and of *Polyporus carneus* Nees, he says

> the wood between the pockets has many hyphae, which pass from one pocket to another.

In his paper on the rot caused in white ash, *Fraxinus americana* L., by *Polyporus fraxinophilus* Pk. (*71*) he finds that "the first hyphae are generally several rings" back from the outer edge of the invasion discoloration areas. Rhoads (*64*) found hyphae in the brown discolored wood (incipient rot) of *Lupinus arboreus* Sims. produced by *Polyporus ostreatus* (Jacq.) Que'l. and *Collybia velutipes* (Curt.) Que'l.

Münch (*58*) found hyphae of *Stereum purpureum* ahead of the brown invasion zone in infected poplar.

Boyce (*9*) in a recent publication states that "Apparently mycelium does not occur in the brown discolored wood in advance of the white spots" in the rot produced by *Fomes fraxinophilus* (Pk.) Sacc. He also states that hyphae are not found in the brown invasion zone of the rot produced by *Fomes igniarius*.

Kauffman and Kerber (*43*) find hyphae and evidence of fungous attack in the apparently sound wood of *Robinia pseudo-acacia* attacked by (*Trametes robiniophila*) (*Polyporus robiniophilus* (Murr.) Lloyd).

MICROSCOPICAL EXAMINATIONS.—The extent to which the hyphae advance in and beyond the discolored areas of incipient rot, is shown by microscopical examinations, and is given in Table II.

Evidently, in certain rots the wood apparently sound for some distance beyond the edge of the discolored areas contains abundant hyphae. The face of the railroad tie section shown in Figure 3, A, when tested by culturing gave positive results from the greater part of the area which appeared entirely normal in texture and color. In a *Picea sitchensis* trunk split open for study the yellow spires, which indicate the farthest limit of the incipient stage of rot, extended up in the trunk 5 feet beyond the last signs of typical rot and for 30 inches beyond the last brownish discoloration. Hyphae were observed in a sample taken from the yellow spires 25 inches beyond the last faint brownish-colored areas. The hyphae of *Polyporus balsameus* in *Abies balsamea* are very numerous in the normal appearing wood several inches beyond the edge of the typical rot area (fig. 3 E). A somewhat similar condition is observed for *Trametes carnea* and *Lenzites sepiaria* in *Picea canadensis*. The longitudinal extent of the hyphae of these fungi beyond the discolored areas has not yet been satisfactorily worked out. The invasion zone of *Fomes igniarius* in *Populus tremuloides* shows hyphae present, although not in large numbers (fig. 3, B). In some collections of this rot showing a faint yellow area bordering the invasion zone hyphae are rarely found, and cultures from this region are usually negative. It is not known whether the hyphae are present and are merely difficult to distinguish or whether the discoloration has preceded the hyphae.

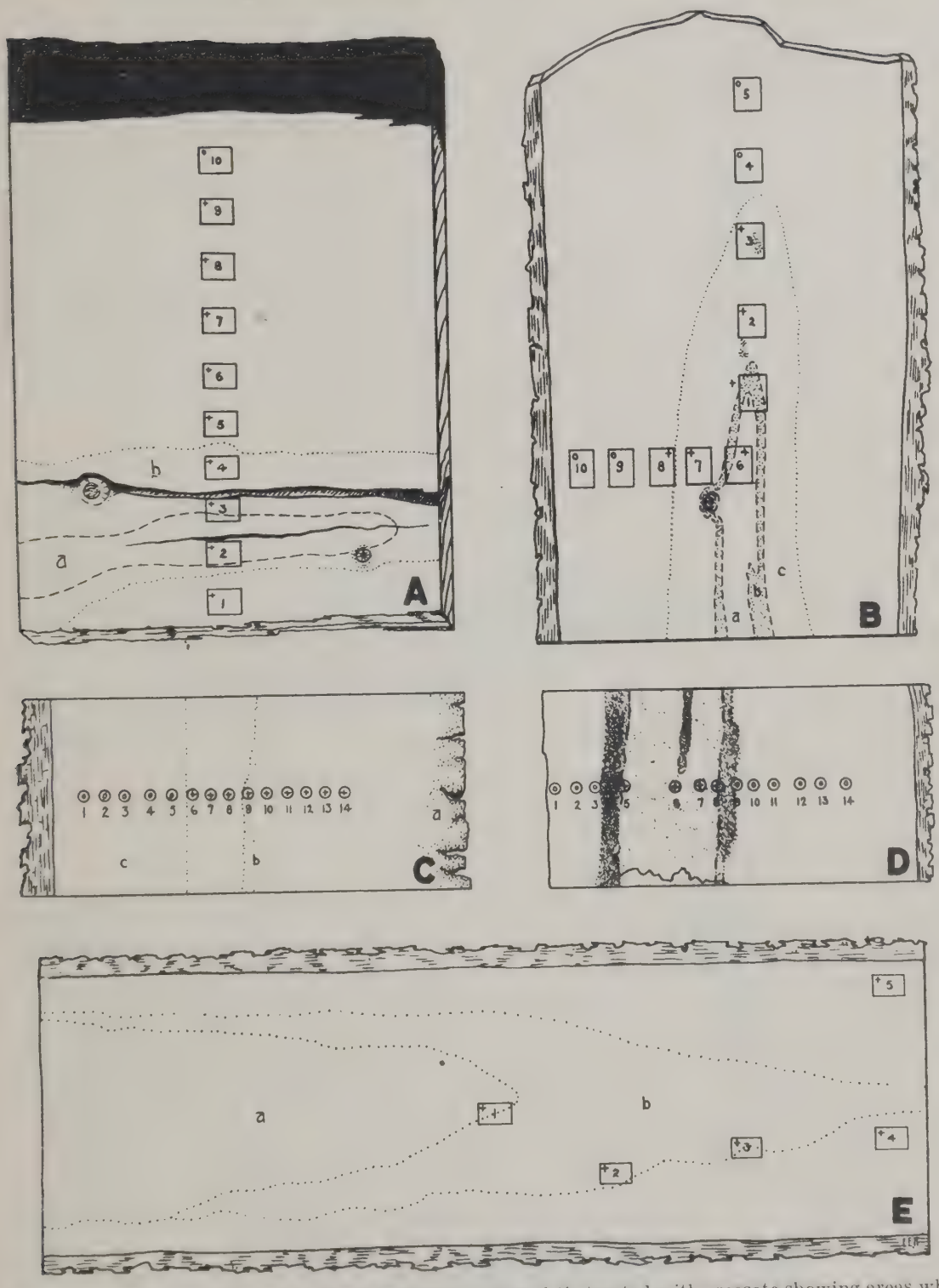


FIG. 3.—A.—Section of jack pine (*Pinus banksiana*) railroad tie treated with creosote showing areas where sections were taken for microscopical examination. Area *a* is the typical stage of brown rot produced by *Lentinus lepideus*, which apparently entered the tie after it was laid in the road bed. Area *b* represents the very faint yellowish-brown area surrounding the typical rot. The remaining area up to the creosoted portion is normal in color. Presence of hyphae is indicated by +, hyphae not observed by *O*. The distance from the edge of the plainly visible color in *A* to the center of No. 9 is $3\frac{2}{16}$ inches. *B*.—Section of young tree of *Populus tremuloides* infected with *Fomes igniarius*. *a*, typical stage of rot; *b*, incipient rot; *c*, very faint yellowish discoloration bordering on *b*. Hyphae were observed in 1 to 7 and in the margin of 8. From edge of area *b* to nearest edge of 8 is three-fourth inch. From edge of *b* to edge of *c*, longitudinally, is $2\frac{1}{16}$ inches. C.—Section from living tree of *Abies balsamea* infected with *Polyporus balsameus*. Typical rot at *a*, incipient rot at *b*, with edge of faintest discoloration shown by dotted line. Sapwood region at *c* limited by straight dotted line. Cultures were made as indicated by numbers and positive results obtained from numbers 6 to 14, inclusive. Cultures from 6, 7, and 8, taken from apparently sound wood, are positive; cultures taken from the sapwood region are negative. No hyphae were observed in the sapwood. D.—Section of young tree of *P. tremuloides* infected with *F. igniarius*. Light shading represents typical rot, heavy shading the brown invasion zone. A zone line is shown at the bottom. Positive cultures were secured from the typical rot area and from one fragment taken from the invasion zone area. The areas showing no discoloration gave negative cultures. E.—Section from living tree of *Abies balsamea* infected with *P. balsameus*: *a*, buff yellow to brownish typical rot area, *b*, incipient faintly colored rot area. Sections for examination were cut as indicated. Hyphae were observed in all the sections and were fairly numerous in 4 and 5 which were taken from the normal colored wood. Distance from 1 in typical rot to 3 in incipient is $2\frac{15}{16}$ inches. Distance from edge of *b* to center of 5 is $1\frac{3}{4}$ inches.

TABLE II.—*Results of microscopical examinations of sections from various stages of rot and from apparently sound areas in wood infected by certain wood-destroying fungi*

[+ indicates hyphae observed; 0 indicates no hyphae observed]

Fungus	Host	Apparently sound; normal color	Incipient rot			Typical rot		Distance in inches from outer edge of discoloration to farthest advanced hyphae	
			Faint color	Discolored	Edge of pocket	Center of pocket	Badly rotted	Hyphae ahead of distinct discoloration	Hyphae within discoloration
Echinodontium tinctorium	*Tsuga heterophylla*	+	+	+			+	Radial 7/8, longitudinal	
Fomes applanatus	*Populus grandidentata*	+		+			+		
Fomes fraxinophilus	*Fraxinus americana*	0	+	+			+	0	1/8 radial.
Fomes igniarius	*Populus tremuloides*	0	+	+			+	Radial 3/4, longitudinal $2\frac{1}{16}$	
Do	*Acer rubrum*	0	(a)	+			+	0	Up to edge of darker zones.
Fomes laricis	*Pseudotsuga taxifolia*	+	+	+			+		
Fomes pinicola	*Tsuga canadensis*	0	+	+			+	Radial 1 1/2, longitudinal	
Fomes roseus	*Pinus banksiana*	[b] +	+	+			+	Longitudinal	
Ganoderma tsugae	*Tsuga canadensis*	0	[c] +	+			+	Radial	
Lentinus lepideus	*Pinus banksiana*	+	+	+			+	$3\frac{2}{16}$ radial	
Lenzites sepiaria	*Picea canadensis*	0	+	+	+	+	+	$2\frac{1}{8}$ radial	
Pholiota adiposa	*Tilia americana*	0	+	+			+	0	Up to or nearly to edge.
Polyporus adustus (?)	*Liquidambar styraciflua*	0	+	+			+	0	Up to edge.
Polyporus anceps	*Picea canadensis*	0	+	+	+	+	+	0	Do.
Polyporus balsameus	*Abies balsamea*	+	+	+			+	Radial 1 3/4, longitudinal $12\frac{15}{16}$	
Polyporus ellisianus	*Pinus ponderosa*	0	+	+	+	+	+	0	Do.
Polyporus schweinitzii	*Picea sitchensis*	+	+	+			+	Radial 3 1/2, longitudinal 25 beyond pale brownish color.	
Polyporus sulphureus	*Pseudotsuga taxifolia*	0	+	+			+	0	Do.
Trametes carnea	*Picea canadensis*		+	+			+	Radial 2 1/8	
Trametes pini	*Pinus monticola, Pinus banksiana, Pseudotsuga taxifolia,* and *Picea canadensis.*	0	0	+	+	+	+	0	Do.

[a] Yellow brown.

[b] Very faint color, pale greenish at edge.

[c] Water-soak.

The presence of hyphae in the various areas in infected wood was determined by microscopical examination of radial and transverse sections specially stained for this purpose (figs. 3 and 4). The Bismarck brown-methyl violet method of staining (*38*) was used in the majority of cases. Plate 5 shows a radial section of *Picea sitchensis* containing minute hyaline hyphae of a wood destroyer, *Polyporus schwenitzii*, stained by the above method. The minute hyphae could not be observed in the wood cells before staining. The piece of wood from which this section was cut showed no discolorations to indicate the presence of a wood-destroying fungus.

Hyphae were noted in the apparently sound wood of *Pinus banksiana* infected with what appeared to be *Fomes roseus*. On close observation this area showed a very faint greenish color on the outer edges and a pale yellowish-brown color between this and the typical rot area.

Hyphae are present some distance beyond the discolored areas in wood infected with *Fomes laricis* and *Fomes pinicola*. The incipient decay produced in firs and hemlocks by the Indian paint fungus, *Echinodontium tinctorium*, is known to continue longitudinally about 4 to 6 feet ahead of the discolored areas. Microscopical examination discloses hyphae some distance radially from the faintest discolorations (fig. 4, B).

Microscopical evidence therefore indicates that in certain rots the hyphae advance as far as the edge of discoloration; and that in other rots, principally those of the brown rot group, the hyphae may be found a considerable distance beyond the faint discolored areas in the incipient rot region.

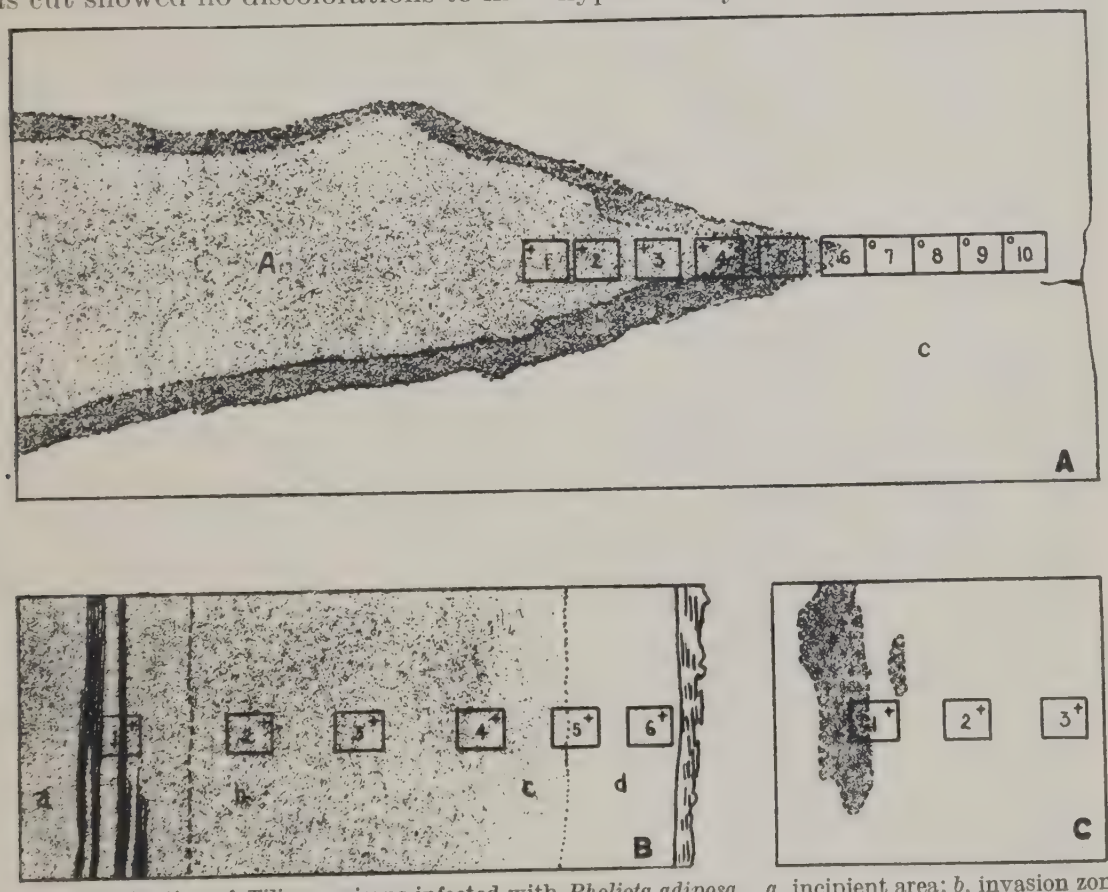


FIG. 4.—A.—Section of *Tilia americana* infected with *Pholiota adiposa*. *a*, incipient area; *b*, invasion zone; *c*, sound area. Sections for examination were taken from areas 1 to 10. Failure to find hyphae in 6 may have been due to the minuteness of the threads. No hyphae were observed in 7 to 10. Cultures made by using fragments from the invasion zone usually give positive results. B.—Section of fallen trunk of *Tsuga heterophylla* infected with *Echinodontium tinctorium*. Typical rot at *a* showing characteristic reddish streaks. Incipient rot at *b* with extremely faint discoloration at *c*. Apparently sound sapwood region at *d*. Hyphae were observed in all of sections 1 to 6. Apparently the fungus invaded the sapwood after the tree died. C.—Section of pulp log of *Picea canadensis* infected with *Lenzites sepiaria*. Typical rot in shaded areas. Very faint discoloration immediately surrounding the typical rot. Region near 2 and 3 apparently sound. Hyphae were observed in all the sections cut for examination. The distance from edge of typical rot to outer edge of 3 is 2⅛ inches.

CULTURAL EXPERIMENTS.—Fragments of infected wood were removed at regular intervals from the typical stages of rot, through the incipient stages and from the apparently sound wood beyond.[3] In the late typical stage of most of the brown rots it was very difficult to obtain cultures uncontaminated by molds. Apparently the shrinkage in this stage of decay

[3] The technique employed was essentially thatdescribed in U. S. Dept. Agr. Bul. 1262 : 40.

allows secondary fungi to enter more easily through the cracks. Table III shows the results of these cultural experiments. Figure 3, C and D, shows how the selection of inoculum fragments is made to extend from the typical decay to the sound areas. As a general rule the apparently sound regions beyond the incipient discolorations in the brown rots contain viable hyphae.

TABLE III.—*Distribution of hyphae of various fungi in infected wood as determined by various cultures*

[+ indicates positive cultures; 0 indicates negative cultures]

Fungus or rot	Host	Apparently sound; normal color	Incipient rot			Typical rot	
			Faint color	Discolored	Edge of pocket	Center of pocket	Badly rotted area
Ceratostomella	*Pinus strobus*	[a] +	+	+			
Echinodontium tinctorium	*Tsuga heterophylla*	+	+	+			+
Fomes applanatus	*Acer saccharum*	0	+	+			+
Do	*Populus grandidentata*	0	+	+			+
Fomes fraxinophilus	*Fraxinus americana*	0	+	+			+
Fomes igniarius	*Populus* sp	0	[b] 0	+			+
Do	*Acer rubrum*	0	0	+			+
Do	*Prunus emarginata*	0		+			+
Do	*Populus tremuloides*	0	0	+			+
Fomes laricis	*Pseudotsuga taxifolia*	0	+	+			+
Fomes pinicola	*Tsuga heterophylla*	0	+	+			+
Do	*Tsuga canadensis*	0	+	+			+
Fomes roseus	*Picea canadensis*	+	+	+			+
Do	*Pseudotsuga taxifolia*	+	+	+	+	+	+
Ganoderma tsugae	*Tsuga canadensis*	0	[c] +	+			+
Do	*Tsuga heterophylla*	0	+	+			+
Lentinus lepideus	*Pinus banksiana*	+	+	+			+
Lenzites sepiaria	*Picea canadensis*	0	+	+	+	+	+
Pholiota adiposa	*Tilia americana*	0	[d] +	[e] +			+
Polyporus adustus	*Liquidambar styraciflua*	0	+	+			+
Polyporus amarus	*Libocedrus decurrens*	0			+	+	
Polyporus anceps	*Picea canadensis*	0	+	+	+	+	
Do	*Pinus banksiana*	0	+	+			+
Polyporus balsameus	*Abies balsamea*	+	+	+			+
Polyporus ellisianus	*Pinus ponderosa*	0	+	+			+
Do	do	0	+	+		+	[f] +
Polyporus schweinitzii	*Picea sitchensis*	+	+	+			+
Do	*Larix decidua*	+	+	+			+
Do	*Pseudotsuga taxifolia*	+	+	+			+
Polyporus sulphureus	*Quercus* sp	0	+	[g] +			+
Do	*Pseudotsuga taxifolia*	0	+	+			+
Schizophyllum commune	*Betula papyrifera*	+	+	+			
Trametes carnea	*Picea canadensis*	+	+	+	+	+	+
Trametes pini	*Pinus banksiana*	0	0	+	+	+	+
Do	*Pseudotsuga taxifolia*	0	0	+	+	+	+
Do	*Pinus monticola*	0		+	+	+	
Do	*Pinus strobus*	0	+	+	+		+
Do	*Larix laricina*	0	+	+	+		+
Trametes serialis	*Pseudotsuga taxifolia*	0	+	+	+	+	
Brown butt rot; pocket rot.	*Thuja plicata*	0		+	+	+	+
Brown cubical rot	*Thuja occidentalis*	0	+	+	+	+	+

[a] White area adjacent to stained area. [b] Yellow. [c] Water-soak. [d] Red brown. [e] Black brown. [f] Rare. [g] Brownish.

Table III shows 8 of the fungi with positive cultures from the normal colored areas, 22 from the faint colored areas, 24 from the discolored areas, 9 from edge of pocket, and 24 from the typical rot areas. Only one case (*Polyporus ellisianus*) did not give cultures from the late typical stage of rot. The results with *Trametes pini* are interesting and indicate that hyphae do not develop beyond the outer edge of the incipient discolorations. This also appears to be true for *Polyporus anceps* in *Pinus ponderosa* and *Pinus banksiana*, and for *Polyporus circinatus* in *Picea canadensis*. The point to be emphasized here is that the hyphae of certain fungi, many of which produce brown rots, do extend some distance beyond the definitely discolored areas which are usually recognized as stages of incipient rot. The average distance to which these hyphae penetrate ahead of the discolorations in each case can be obtained only after many tests of freshly cut material.

THE EFFECT OF HYPHAE ON THE WOOD TISSUES

METHODS OF CELL WALL PENETRATIONS.—One of the earliest writers to figure the penetration of cell walls by fungous threads was Unger (*80*), who clearly showed the constriction of the hyphae in passing through the bore

hole. The first reference to wall penetration by wood-rotting fungi is given by Willkomm (*90*), who made several excellent drawings showing the hyphae in the wood tissues but showed no definite penetration of the hyphae through the cell walls. R. Hartig (*28, 29, 30*) was the first to demonstrate and illustrate correctly the penetration of cell walls by hyphae of wood-rotting fungi. Since Hartig's time comparatively little has been added to our knowledge of the subject. His work (*28*) shows that there are two principal methods of penetration exhibited by the various fungi studied. The (older) hyphae of the one group are always constricted when passing through the cell walls, while those of the other group show no constriction but pass freely through an opening in the cell wall as large or larger than the hyphal thread.

Hartig illustrates the larger hyphae of *Armillaria mellea* (*28, Pl. 11*) greatly constricted, with the small, apparently young hyphae showing no constriction. He shows the hyphae of *Polyporus borealis* markedly constricted (*28, Pl. 10*), with no enlargement near the host cell walls. But he figures the hyphae of *Polyporus fulvus* (*28, Pl. 7*) passing through the cell walls by way of large hourglass-shaped bore holes, and *Trametes pini* (*28, Pl. 6*), *Polyporus schweinitzii* (*28, Pl. 9*), and *Fomes igniarius* (*28, Pl. 16*) passing through cell walls without constriction. A few bore holes are shown with no hyphae passing through in *Stereum hirsutum* (*28, Pl. 18*). For *Fomes annosus* (*Trametes radiciperda*) a few bore holes and one indistinct penetration are noted (*28, Pl. 4*). No penetrations by hyphae are shown for *Polyporus sulphureus* (*28, Pl. 14*).

Lindroth (*45*) records the hyphae of *Polyporus nigricans, Polyporus betulinus,* and *Polyporus laevigatus* boring without constriction through the cell walls.

White (*89*) notes the constriction of hyphae of *Fomes applanatus* in infected wood. Hiley (*35*) records constriction of hyphae for *Dasyscypha calycina, Fomes annosus,* and *Polyporus schweinitzii* in larch wood. In the latter fungus the constriction is marked and in *Armillaria mellea* the hyphae are of the same size as the bore holes. These statements do not correspond with Hartig's records as given above.

Buller (*14, fig. 1*) illustrates the penetration of tracheid walls of pine by hyphae of *Lentinus lepideus.* No constriction is shown and the bore holes are larger than the hyphae.

The mechanics of penetration through plant cell walls of hyphae of various fungi have always interested pathologists. There are several possible ways. Openings already provided may be used (*36, 57, 72*), or by means of enzymic action (*3, 20, 26, 35, 42*) an opening may be dissolved out of the wall. A third possibility is the forcing of an opening by mechanical means (*31, 32, 52, 55*). Hawkins and Harvey (*32*) give a résumé of the literature on penetration of plant cell walls and conclude that some good evidence supports the view that certain parasites penetrate the hosts by mechanical means.

Many wood-inhabiting fungi pass from one cell to another through such natural openings as the simple or bordered pits. It is of interest to note that the hyphae of wood-destroying fungi as a rule do not seek these natural openings but pass through the cell walls at random, often passing through a large number of cell walls in a direct line, and often passing through the bordered pit very near its opening. In the case of wood-rotting fungi it would seem that the greater resistance offered by the thick, lignified walls of the tracheid and wood-fiber cells would preclude any possibility of a fungus hyphae forcing an opening by mechanical means only. The theory of enzyme action more nearly fits the facts observed.

Size and shape of bore holes.—The bore holes by which the hyphae pass through the cell walls vary considerably with the stage of the rot and with the fungus in question. In certain rots they are small in the incipient stage and become larger in diameter as the decay progresses (figs. 5 and 6).

In such rots as those produced by *Trametes pini,* where the hyphae are constricted, very little change in the diameters of the bore holes is noted (fig. 5, B; fig. 6, 1 to 12). Those through which the very young hyphae pass are rarely smaller in diameter than those through which the older and larger hyphae extend. Where constrictions occur in the hyphae when passing through the cell walls the bore holes are usually even, cylindrical, and perpendicular to the surface of the walls. This is brought out very well when the holes are seen in longitudinal section (fig. 5, Be and Fd).

In other rots the section view shows the bore holes with irregular outlines, in many cases resembling roughly an hourglass. This feature is always found in connection with bore holes which are usually twice to several times larger in diameter than the

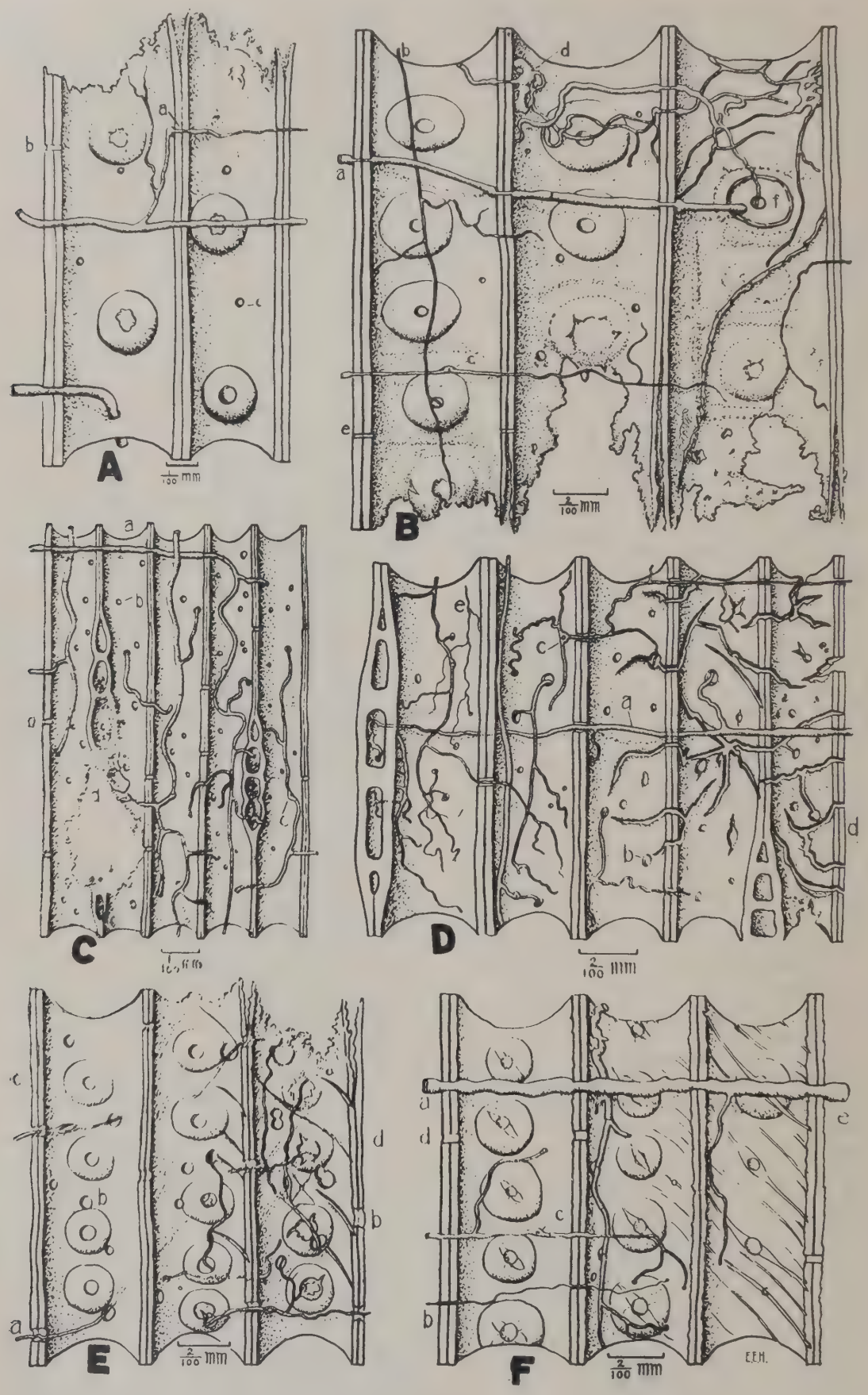


FIG. 5

FIG. 5.—A.—*Polyporus balsameus* in *Abies balsamea*. Typical stage of rot. Radial section through heartwood. *a*, Constriction of hyphae in passing through cell wall. *b*, *c*, bore holes produced by hyphae. B.—Radial section through heartwood of *Pinus strobus* infected with *Trametes pini*. Section taken near edge of white pocket. *a*, An older hypha of a brownish color showing the habit of constriction when passing through the cell walls; *b*, a young hypha, hyaline, and branched (the constriction of the thread does not appear in these young hyphae); *c*, characteristic clamp connection often found in wood infested by this fungus; *d*, tangled masses of apparently anastomosed hyphae; *e*, bore hole with fungus thread dropped out; *f*, embossed portion of bordered pit entirely decomposed. The effect on the middle lamella allows a separation of the individual cell walls. *C*.—Tangential section through the heartwood of *Tsuga heterophylla* infected with *Echinodontium tinctorium*. Section from typical stage of the rot. The general character of the dissolution of cell walls may be noted. *a*, an older hypha penetrating several cells walls and showing slight constrictions on passing through the bore holes; *b*, bore holes made by the hyphae in passing through the cell walls; *c*, characteristic clamp connection not often observed in the decayed wood; *d*, dissolution of the medullary ray and portions of the lignified cell wall of the tracheid. D.—Tangential section through the heartwood of *Picea canadensis* infected with *Trametes carnea*. Section taken from the edge of typical stage of the rot. *a*, hypha penetrating several cell walls and showing no constriction in passing through the walls. In this case the bore holes are usually much larger than the hyphae; *b*, characteristic bore hole with hyphal thread dropped out; *c*, this type and the simple type of clamp connection are frequently observed in the infected wood; *d*, peculiar uncommon form of shrinkage cracks found in the typical stage of rot. The spiral form of shrinkage crack is absent; *e*, very minute hyaline hyphae are often observed. The bore holes produced by these hypha are also larger than the hyphae. E.—Radial section through the heartwood of *Pinus banksiana* infected with *Lentinus lepideus*. Section from early typical stage of the rot. *a*, Hyphal thread penetrating tracheid cell wallse one of the very large and characteristic bore holes is shown at *b*, where the irregular surface of the bor; can be noted; *c*, a peculiar type of medallion or detour hypha commonly found in this material; *d*, spiral shrinkage cracks ascending from right to left and extending as far as the middle lamella of the cell wall. F.—Radial section through the heartwood of *Libocedrus decurrens* infected with *Polyporus amarus*. Section from the edge of the pocket of brown cubical rot. Hyphae are generally scarce in this rot. *a*. Large hyaline hypha penetrating several tracheid cell walls which show roughened surfaces due to the action of the fungus (the hypha is constricted in its passage through the walls); *b*, minute hyaline hyphae are occasionally observed and these appear to be slightly smaller than the bore holes they produce; *c*, hypha showing clamp connection; *d*, bore hole, showing the even smooth surface; *e*, spiral shrinkage cracks ascending from right to left are common in the typical stage of the rot and appear to be more numerous in the cells of the summer wood. The bordered pits show evidence of the fungus action and cases may be noted where little is left to indicate the original location of the bordered pit.

FIG. 6.—Penetration of hyphae of *Trametes pini* through tracheid cell walls of *Pinus strobus* is shown in 1 to 12. (The older, larger, brown hyphae in 1 diminish in size to the small hyaline hyphae shown in 10.) An oblique cut through hypha and bore hole, rather than the early stage of penetration, is shown in 11. Surface view of a hypha passing through a bore hole is shown in 12. The diameters of the bore holes vary but little. Radial section of *Pinus ponderosa* infected with *Polyporus anceps* (*P. ellisianus*) shown in 13. Incipient stage of rot, showing size of bore hole and constriction of the hyphae. The bore holes made by the very small hyphae seem to be larger than the hyphae. A spore-chain formation of the hyphae is often observed as shown in the drawing. Radial section of *Tilia americana* infected with *Pholiota adiposa* is shown in 14. Incipient stage of rot secured by inoculating a sterilized block with a pure culture of the fungus. The hyphae are slightly constricted in passing through the cell walls. Tracheid wall of *Picea sitchensis* infected with *Polyporus schweinitzii*, from the typical rot area, shown in 15, with penetration by hyphae and types of bore holes. Slight constriction of the hyphae in the larger threads (*d*). The bore holes are irregular and constricted at the middle lamella (*c* and *e*). Bore holes accompanying the very small hyphae seem larger than the threads passing through them. Radial section of *Populus balsamifera* infected with *Fomes igniarius* shown in 16. From the invasion zone area near the edge of the typical rot. *a*, One of the minute hyaline hyphae passing through bore holes in the cell walls of the wood fibers; *b*, one of the larger hyphae passing through the walls. The bore holes are very much larger than the hyphae; *c*, typical bore holes showing the greater resistance to dissolution exhibited by the middle lamella. Radial section of *Pinus banksiana* infected with *Lentinus lepideus* is shown in 17. From the faintly discolored area near edge of typical rot. Young hypha (*a*) and older hypha (*b*) passing through cell walls; *c*, type of bore hole showing constriction at middle lamella; *d*, characteristic "detour" or "medallion" hypha frequently found in this material. Radial section of *Betula papyrifera* infected with *Polyporus betulinus* is shown in 18. From typical rot area, showing penetration, size, and shape of bore holes. Radial section of *Betula papyrifera* infected with *Fomes fomentarius* is shown in 19. From near edge of typical rot area. The bore holes are of typical "hourglass" shape. Radial section of *Tsuga heterophylla* infected with *Echinodontium tinctorium* is shown in 20. From the early typical stage of rot. No constriction of the hyphae nor of the bore holes was observed. *a*, Older hypha with young branch at *b* showing penetration through the pit membrane; *c*, bordered pit showing action of the fungus in dissolving the embossed portion. Radial section of *Robinia pseudoacacia* L. infected with *Trametes robiniophila* (*P. robiniophilus*) shown in 21. From typical stage of rot, showing penetration of the tylose walls in the pore cells by the hypha. The bore holes are larger than the hypha. Semidiagramatic view of the bordered pit in the tracheid wall of *Pinus strobus* is shown in 22, with the hypha of *Trametes pini* passing through the wall at *a* and through the bordered pit at *b*. Older hypha (*a*) and young hypha (*b*) of *Trametes pini* passing through cell walls in *P. strobus* are shown in 23. Radial section of *Libocedrus decurrens* infected with *Polyporus amarus* is shown in 24. From edge of rot pocket. Penetration and characteristic constriction of older hypha and type of bore hole appear.

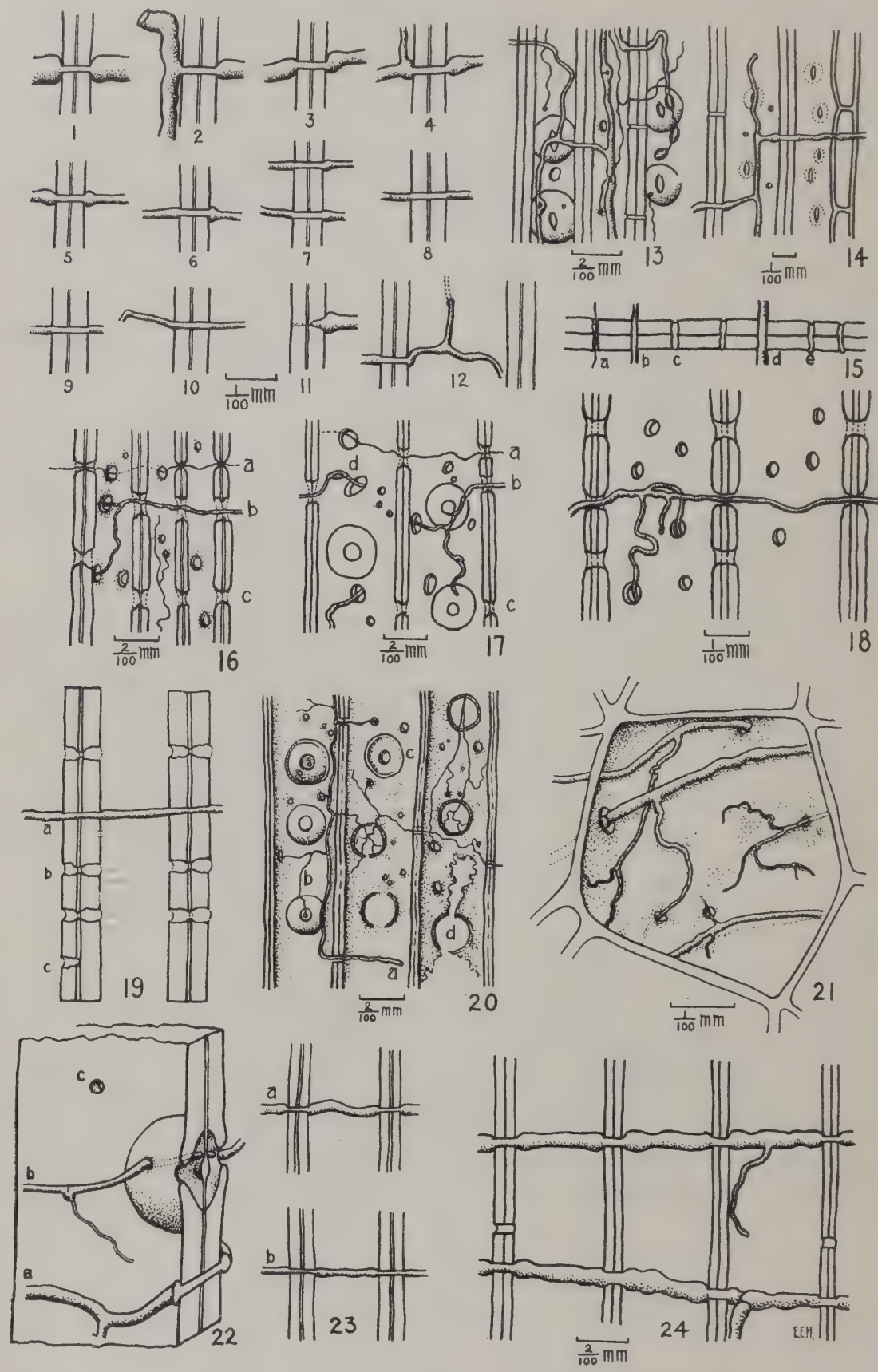


FIG. 6

(For explanatory legend see p. 547.)

hyphae which pass through them (16, 17, 18, 19, in fig. 6). The hourglass shape appears to be caused by the greater resistance of the middle lamella to the decomposition process (19b, fig. 6). In a few cases hyphae were noted passing through bore holes of the same diameter as the hyphae. This is often observed where young hyphae are penetrating the walls (8, 9, 10, in fig. 6). The hyphae pierce any part of the cell wall, often the curved or embossed part, or the membrane of the bordered pit; occasionally they pass through the pit openings (fig. 5, Bf and E).

Hiley (*35*) in discussing the hyphae in larch wood infected with *Fomes annosus* states that—

> to make these bore holes, the hyphal tips must excrete the necessary enzymes (presumably a lignase and a cytase). When once a hypha has gained passage from one tracheid to the next, the hole which it occupies ceases to enlarge, and it may reasonably be deduced from this that the enzymes are only secreted by the apices of the hyphae.

Observations on a number of fungi causing wood rot, and careful comparison of the very young hyphae with the older ones which showed constrictions, indicated that the bore holes were usually as large or only slightly larger in diameter than the diameter of the young hyphae found penetrating the cell walls. The relative size of bore holes in the tracheid cell walls in *Pinus strobus* infected with *Trametes pini* and of the hyphae producing them is shown in 1 to 10 of Figure 6. The bore hole produced by the largest hypha (fig. 6, 1) is little different in size from that produced by a very young hypha (fig. 6, 10), exhibiting no pronounced constriction. In (fig. 6, 11) is shown what might be taken for the early stage of penetration of a hypha, which is interpreted as the effect produced by sectioning obliquely through the hypha and the bore hole. Numerous hyaline hyphae smaller than those shown in 9 and 10 of Figure 6 were noted in the tracheids, but they were not observed penetrating the walls. Some idea of the number of bore holes in the infected tissues may be gained by the photomicrograph in Plate 7 showing *Trametes pini* in southern yellow pine. Apparently, the young hyphae can only dissolve the cell wall, and beyond a certain point are incapable of enlarging the bore holes. The hyphae continue to grow in diameter and the characteristic constrictions are produced where the hyphae pass through the bore holes in the cell walls. All the evidence appears to confirm Hiley's observations on *Fomes annosus*.

Absence of hyphae in certain stages.—Very often when prepared sections, particularly those cut from the typical stage of a brown rot, are examined under the microscope, numerous bore holes and other evidence of fungus action appear but hyphae are absent. It is not clear whether absence of hyphae is due to loss during the handling of sections, or to resorption of the old hyphae, or, in the case of incipient stages of rot, to the invisibility of very fine threads (Pl. 5) (*43*).

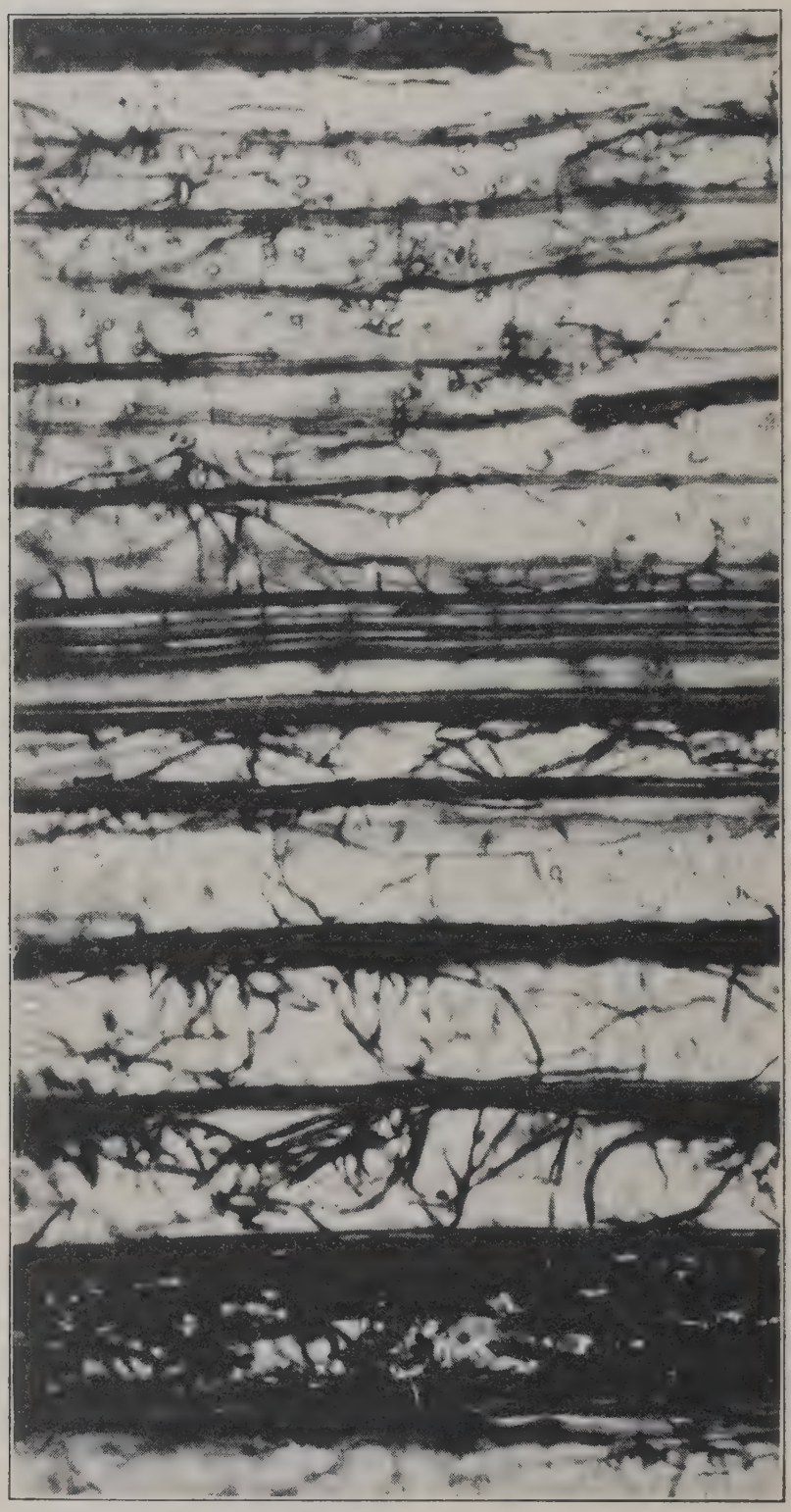
Buller (*11*, *14*) states that in wood decayed by *Lentinus lepideus* the hyphae often disappear, leaving but a trace of their former existence in the form of hyphaelike deposits of calcium oxalate crystals.

Rhoads (*63*) finds scanty mycelium in the typical stage of rot produced by *Polystictus pargamenus*, and notes this peculiarity in other rots. He suggests that the enzymes produced by the fungus dissolve the older hyphae.

Kauffman and Kerber (*43*), working with the rot produced in black locust by *Trametes robiniophila*, had difficulty in finding hyphae of the fungus in the older stages of decay, though bore holes and other signs of fungus activity were evident. They find this a frequent phenomenon in the typical stages of rot caused by some of the common wood-destroying fungi.

Since bore holes vary for different rots (see above, p. 545), and certain infected woods exhibit bore holes considerably larger than the hyphae which pass through, it is possible that in cutting microtome sections from such material without embedding in celloidin or paraffin the hyphae may become broken and may eventually become lost from the section.

A summary of the main microscopical characters of diagnostic value assembled during this study is given in Table IV. In the absence of hyphae identifiable as wood destroyers the bore holes are perhaps of prime importance. Spiral cracks and corrosion marks are reliable when accompanied by hyphae, particularly when the hyphae show no characters such as buckles or medallions which can be used to class them as wood-rot fungi. In wood showing no distinctive gross or microscopical characters the proof that the hyphae observed belong to a wood-rot fungus rests entirely upon the success of cultural methods. (These observations may not hold for all species of wood and under all conditions. The number of species examined is limited, particularly for any given fungus, and the study should be continued to obtain further



The Diagnosis of Decay in Wood **PLATE 7**

Photomicrograph of a tangential section of southern yellow pine infected with *Trametes pini*. Note the numerous bore holes produced in the tracheid cell walls by the hyphae. ×250.

TABLE IV.—*Diagnostic characters of various fungi causing wood rots.—Observations based mainly on typical stage of rot*

Fungus	White rot	Brown rot	Zone lines	Bore holes large	Bore holes small	Spiral cracks	Old hyphae not constricted [a]	Old hyphae constricted [a]	Old hyphae with little or no constriction	Buckles noted	Medallion hyphae	Hyphae beyond incipient color
Armillaria mellea	×		×		×			×	×	0		
Echinodontium tinctorium		×	×	×			×			×		×
Fomes annosus	×				×			×		0		
Fomes applanatus	×		×		×			×				×
Fomes connatus	×		×	×			×			[b]×		
Fomes everhartii	×		×									
Fomes fomentarius	×		×	×			×			0		
Fomes fulvus		×	×	×		×	×					
Fomes fraxinophilus	×		×	×			×			×		
Fomes igniarius	×		×	×			×			0		
Fomes laricis		×		×		×	×			×		×
Fomes nigrolimitatus	×		×		×				×	0		
Fomes pinicola		×		×		×	×			×		×
Fomes roseus		×		×		×	×			×		×
Ganoderma tsugae	×		×				×	[c]×	×	×		
Ganoderma curtisii	×		×							0		
Hydnum septentrionale	×		×	[d]×			×			0		
Hymenochaete rubiginosa	×		×	[d]×	[e]×		×		[e]×	×	×	×
Lentinus lepideus		×		×		×	×					
Lenzites betulina		×		×		×	×			×	×	
Lenzites sepiaria		×		×		×	×					
Merulius lachrymans		×		×						0		
Pholiota adiposa		×			×	×		×		0		
Pleurotus ulmarius		×		[b]×	[e]×		[f]×	[e]×		0		
Pleurotus ostreatus	×			×			×			×		
Polyporus adustus (in *Populus* sp.)	×			×			×					
Polyporus amarus		×			×	×		×		×		
Polyporus anceps	×				×			×				
Polyporus balsameus		×		[g]×	×	×	[g]×	×		[g]×		×
Polyporus berkeleyi		×		×			×					
Polyporus betulinus		×		×		×	×			0		
Polyporus borealis	×				×			[b]×		0		
Polyporus circinatus	×			×			×			0		
Polyporus guttulatus		×		×		×	×			0		
Polyporus resinosus	×		×	×			×			0		
Polyporus robiniophilus	×		×	×			×			0		×
Polyporus schweinitzii		×			×	×		[h]×	×	0		×
Polyporus squamosus	×			×			×			×		
Polyporus sulphureus		×		×		×	×			×		
Polystictus abietinus	×		×	×			×			×		
Polystictus hirsutus	×			×			×			0		
Polystictus pargamenus	×		×	×			×			×		
Polystictus versicolor	×		×	×			×			0		
Poria incrassata		×	×	×		×	×			0		
Poria laevigata	×			×						0		
Poria subacida	×				×			×		×		
Stereum sulcatum	×		×		×			×		0		×
Trametes carnea		×		×		×	×			×		
Trametes malicola		×		×		×	×					
Trametes protracta		×		×		×	×			×	×(?)	×
Trametes serialis		×		×			×			×		
Trametes pini	×		×		×			×		0		
Trametes isabellina	×		×	[f]×			[f]×			0		
Xylaria polymorpha	×		×	[f]×	[e]×			[e]×				

[a] Passage of hyphae through cell walls of host.
[b] Rare.
[c] In summer wood.
[d] In vessels.
[e] In fibers.
[f] Pitted tubes.
[g] Late typical stage.
[h] Only when passing through heavily lignified walls. (Buller.)

data.) Table IV shows that large holes are more commonly found in the brown rots than in the white rots (with a notable exception in *Polyporus schweinitzii*). The white pocket rots so far studied, as a rule show small bore holes and constricted hyphae. Spiral shrinkage cracks appear to be exclusive features of the brown rots and apparently the enzymes of the causal fungi have here played an important part. The fact that brown rot fungi can apparently remove from the cell wall much of the so-called cellulose complex, leaving most of the lignin compound (see under "Decay processes" above), may be a clue to the origin of these cracks. May not the cracks, forming at the weakest tension point, follow the hollows between the spiral thickenings laid down in the formation of the tracheid cell wall? Another question is suggested: Do shrinkage cracks appear only in the wood tissues infected by fungi or are they found in sound tissues as well? Upon this answer hinges the diagnostic value of shrinkage cracks in wood tissues where hyphae are not observed. It is not inconceivable that the enzymes of a wood-rotting fungus may affect the tissues ahead of the hyphae and so produce shrinkage cracks in tissues where hyphae are lacking(*19, 26, 35, 51, p. 379*).

As an example of the value of microscopical characters in decay diagnosis, the differences between *Trametes pini* and *Polyporus circinatus* may be cited. The hyphae of *Trametes pini* in *Picea canadensis* are constricted in passing through the comparatively small bore holes in the cell walls and are commonly seen to penetrate in a direct line across the section through many walls. Buckles are observed. The hyphae of *Polyporus circinatus* in *Picea mariana* are not constricted, and the bore holes are usually much larger than the hyphae passing through them. The majority of the hyphae run lengthwise of the long axis of the cell. Only a few penetrate many cell walls in a direct line, and none show buckles.

Again, in the incipient stage of *Echinodontium tinctorium* in *Tsuga heterophylla* bore holes and hyphae are numerous and the surfaces of the cell walls appear slightly pitted or corroded. Th hyphae penetrate the tracheid cell wall and pass through the bordered pits, in places filling the cell cavities with masses of branched and twisted hypha . The hyphae have a tendency to concen trate in the medullary ray cells. N great changes are noted in the cell walls In the typical stage there is a marked dissolution of the cell walls, particularly in the region of the ray cells (fig. 5, Cd.). The decrease in width of the cell walls is marked, the fungus attacking the tertiary wall first, the dissolution progressing toward the middle lamella. In most of the sections examined the middle lamella was apparently left unaffected. The entire cell wall was reduced to a width of 1.5 to 2.5 μ wide as compared to 2.5 to 3.2 μ wide in the normal wood. The walls show no evidence of shrinkage cracks, but grooves which can be distinguished are apparently caused by contact with hyphae on their inner surfaces. A reddish to brownish decomposition by-product is often found filling the tracheid and medullary ray cells.

Following are the results of microchemical tests made on sections of infected wood of *T. heterophylla* and *Abies grandis*. With Millon's reagent a reaction for proteins was obtained in and near the zone lines and the areas containing these by-products. In the typical stage of decay such reagents as chloroiodide of zinc, phloroglucin and hydrochloric acid, potassium permanganate, potassium dichromate, ferric chloride, aniline sulphate and paranitroaniline gave, in general, a reaction for the lignin compounds. The absence of tannin throughout the rotted area was also determined. In the bleached to white patches the reactions indicate that considerable of the cellulose compound is present. With tincture of alkanin slight traces of resin were found near the sapwood region. With iodine a few starch grains were observed in the less decomposed medullary rays.

CULTURAL CHARACTERS OF DECAY

The third step after examination for gross and microscopical characters of decay involves a cultural technique described in an earlier paper (*40*). Long and Harsch have shown (*50*) that cultural methods can aid in identifying the organism causing a specific decay in wood.[4] The cultural data on pure cultures of the fungus isolated from the infected wood and grown upon a suitable standard medium should include observations upon the source of inoculum; the macroscopic and microscopic characters of the mycelium, with emphasis upon secondary spore formation (*76*); the size, color, and character of the hymenial layers produced in the near-typical and typical pilei; and conditions of light and temperature to which the cultures were exposed. To these data may be added proof of the important

[4] An important paper by C. W. Fritz (*25*) has come to the writer's attention since the preparation of the present papers.

question whether the isolated fungus, when placed in contact with sterilized sound wood under favorable conditions, produces a rot identical with that from which it was isolated.

The one difficulty to be guarded against is that of contaminations. Contaminating fungi present in the sample produce mixed cultures of very uncertain value; and the contaminations incidental to the technique of culturing are usually due to fungi which either inhibit or mask the development of wood destroyers on the agar. Mention has already been made of the fact that the late typical stage of the brown rots often shows a large amount of mold contamination when cultured. For critical study such material is obviously objectionable.

For extensive comparison of the color reaction produced by specific fungi, Long and Harsch (*50*) have recommended various media, but in routine commercial diagnosis the use of 8 or 10 different media is impracticable. Plain malt agar slanted in tubes has been found a very satisfactory standard medium in the present cultural work.

ARTIFICIAL PRODUCTION OF TYPICAL DECAYS

White (*89*) has noted the fact that there appears to be one general weakness in method throughout the works on forest pathology. This weakness is the identification of causal agents of wood rots based almost entirely upon the more or less constant association of the fungus with the rotted wood. In plant pathological investigations and in all standard bacteriological research Koch's rules are applied in their entirety in order thoroughly to establish the relationship between the causal organism and the symptoms. The comparison between bacteriological technique and organisms involved and the technique and organisms involved in wood-rot studies can not be drawn too closely. However, in the study of wood rots only the first rule, that of constant association of the organism with the disease, has been applied with any degree of consistency. It is equally important to establish with precision the relation between cause and effect in the study of wood rots as it is in the study of other plant diseases. The fact that more than one organism may be found developing simultaneously in the same substratum is sufficient ground for establishing a more accurate test for the identification of a wood-destroying organism with the particular type of decomposition produced in wood.

Only a few of the papers examined give cultural data showing the relationship between fungus and wood rot. Of these, only the work on *Fomes applanatus* by White (*89*) indicates intentional application of the rules of proof. He inoculated basswood, poplar, pine, spruce, tamarack, hemlock, fir, cedars, maple, and willow blocks with pure cultures of *Fomes applanatus* and secured typical rots in all but the conifers, where slight infection only was noted. Typical sporophores shedding mature spores were produced.

Abbott (*1*) secured the typical decay caused by *Trametes pini* in tamarack, pine, hemlock, spruce, balsam, birch, and oak by inoculation with pure cultures of the organism. The above order of the hosts, according to Abbott, is the order of susceptibility to the fungus.

Bayliss (*4*) produced the typical rot caused by *Polystictus versicolor* Fr. by inoculating various hardwoods with pure cultures of this organism. Typical sporophores were also produced, partly under natural conditions, on the infected blocks. Infection was not secured in the wood of pine and larch.

Zeller (*92*) used *Lenzites sepiaria* to inoculate blocks of southern yellow pine for durability tests and noted that the typical rot was produced in all cases.

The writer has produced incipient and typical decay with a number of wood-rotting fungi developing under artificial conditions. The following method was used in conducting these experiments:

(1) Numerous field observations (extending over several years) were assembled to check the constant association of the organism with the particular wood rot in the typical stage. (2) The organism was isolated from the decayed wood and pure cultures established, using fragments of decayed wood, sporophore tissue, and spores. (3) Sound sterilized wood was inoculated with pure culture inoculum, to prove that the organism can produce the incipient and typical stages of decay. (4) The organism was reisolated from the inoculated wood and compared with the original pure cultures or stock cultures, or with both. In the isolation of the organism from the piece of rotted wood 10 to 12 tubes were prepared from each wood sample (fig. 3, A). The fragments were selected from various areas on the surface representing both incipient and typical stages of decay. Subcultures were then made from these original cultures. Two sizes of sound wood pieces were used, each an inch square, the one being 5½ inches long, the other 11

inches long. Special length test tubes were used with the 11-inch sticks. A plug of cotton 2 inches long was placed in the bottom of each tube, the stick was next inserted and 200 c. c. of distilled water poured in. The tubes were then autoclaved for 45 minutes at 15 pounds steam pressure. The sticks were next inoculated by placing a small fragment of the mycelial growth taken from a pure culture of known fungus upon the stick at a point approximately 5 inches up from the base.

In some cases the inoculum used was from pure cultures secured from spores or from sporophore tissue of the fungus. A piece of waxed paper was next placed over the cotton plug and fastened closely to the tube by means of a strip of heavy gummed paper. Most of the tubes were kept 8 to 12 months or longer before they were opened and the wood examined for results. In a few cases, 6 months or less produced the typical stage of decay in the portion of the stick where the moisture conditions were most favorable to the action of the fungus. At the end of the test, blocks were cut from the test sticks and cultures made from these in the usual manner (*40*). These were carefully compared with the pure cultures previously obtained and with stock cultures of known fungi secured from spores or from sporophore tissues. The results of the tests are given in Table V. Some of the fungi were inoculated on various hosts in order to secure their reactions on each. Of the 29 fungi used in the tests, 2 produced perfect sporophores and 4 produced poroid growths on the infected wood. *Xylaria polymorpha* produced sterile, black, club-shaped growths.

In several cases the preference for the sapwood over the heartwood was quite pronounced and the organism developed rapidly and extensively on the sapwood blocks but barely covered the surfaces of the heartwood blocks (Pl. 9, C and D). The effect upon the wood was found to be in somewhat the same relationship. For instance, *Polyporus adustus* (?), *Fomes igniarius*, *Fomes applanatus*, *Polyporus anceps*, and *Polystictus versicolor* were inoculated on both sapwood and heartwood blocks and all five appeared to develop more rapidly upon the sapwood.

The results secured by inoculating *Tilia americana* with *Zylaria polymorpha* were very striking. The typical white spongy-rot accompanied by a narrow black zone line formed across one end of the block (Pl. 11, A) was disclosed upon splitting open the block. The surface of the block and the cotton in the bottom of the tube were covered in spots with grayish-black mycelial wefts and with the thin black crustlike layers so typical of the roots of *Acer saccharum* infected with this fungus. Sterile fruiting bodies also appeared at the base of the blocks.

The typical brown cubical rots of *Lenzites sepiaria* and of *Polyporus schweinitzii*, charcoal-like in consistency, were produced artificially in blocks of *Picea sitchensis* (Pl. 11, B and C) and of *Lentinus lepideus*, *Fomes laricis*, *Fomes roseus*, and *Fomes pinicola* (Pl. 8, A to H). The rot produced by *Lenzites sepiaria* showed a greater tendency to form numerous shrinkage cracks which divided the rotted wood into small cubes.

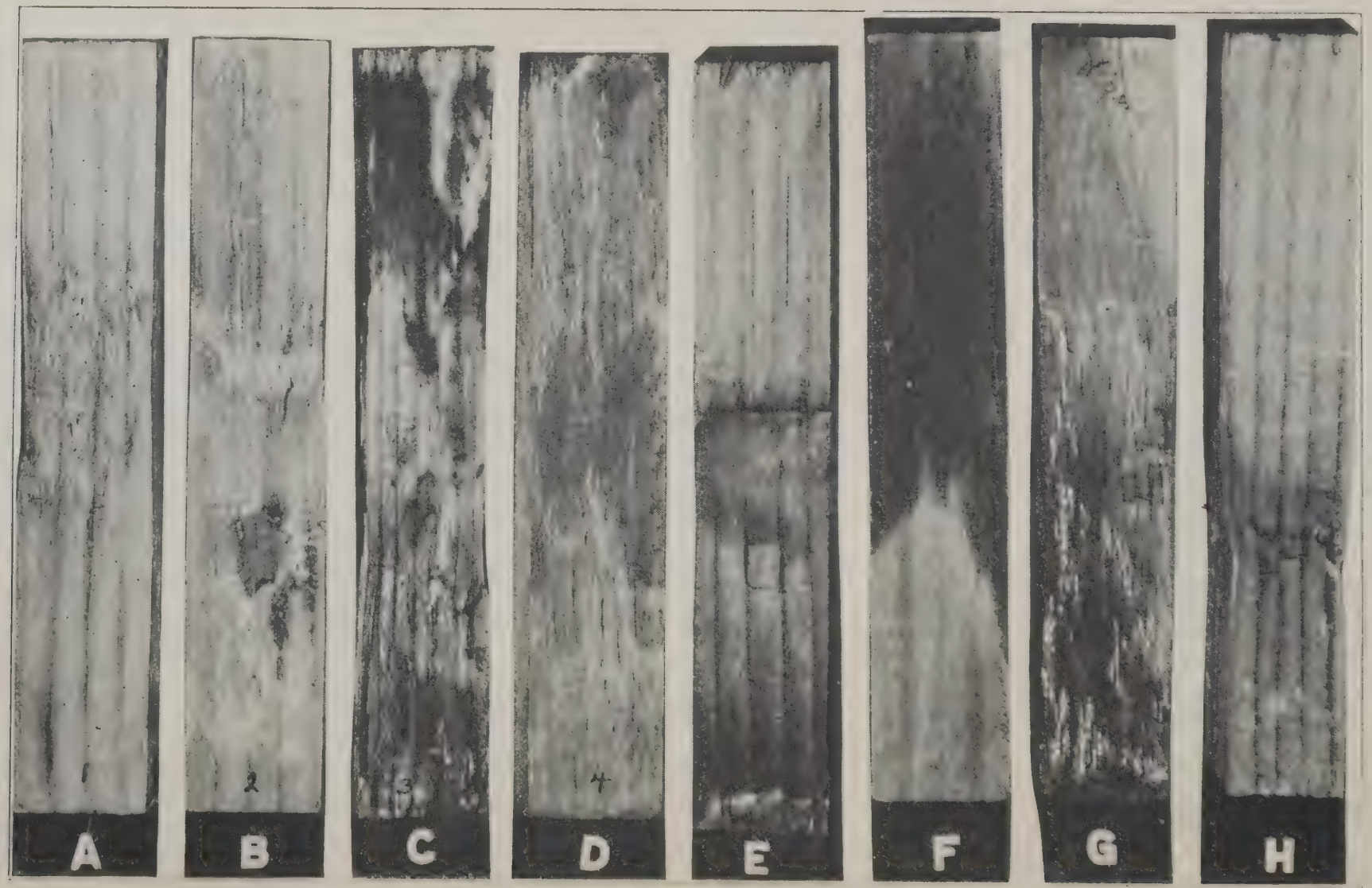
The rot produced artificially in *Picea sitchensis* and *Tsuga heterophylla* by *Trametes pini* is quite typical (Pl. 11, D). White pockets are numerous and typical narrow brownish zone lines are formed across and close to the ends of the pieces. The wood surrounding the pockets is stained reddish to reddish brown.

A valuable cultural character (which unfortunately does not always develop) is the production in pure cultures of near-typical to typical sporophores from which mature spores are often cast (Pl. 10). The sporophores, though diminutive, are frequently characteristic enough for identification and the spore casts furnish a means of checking the purity of the culture, as well as the character of the spores.

A cultural method using sound sterilized wood as a medium and in vitro applicable to the study of unidentified wood rots will be found helpful in verifying the identity of causal fungi in regions where the rots are numerous and where the sporophores rarely develop. In the younger stands of timber old enough to contain rot but too young to bear sporophores of the rot fungi, this type of cultural test will serve as a valuable check of the data obtained from a study of the gross, microscopical, and cultural characters.

EXPLANATORY LEGEND FOR PLATE 8

Brown rots produced by inoculation, showing typical rot with characteristic shrinkage in the rotted areas A and B.—*Fomes laricis*, taken from *Pseudotsuga taxifolia*, on *Picea sitchensis*. C.—*Lenzites sepiaria* on *Picea sitchensis*. D.—*Fomes roseus*, taken from *Picea canadensis*, on *Pinus strobus*. E.—*Lentinus lepideus*, taken from *Pinus taeda*, on *Picea sitchensis*. F.—*Fomes laricis* on *Pinus strobus*. G.—*Trametes carnea* on *Pinus strobus*. H.—*Fomes pinicola*, from *Tsuga heterophylla*, on *Picea sitchensis*.—Eight-elevenths natural size.



(For explanatory legend see p. 554)

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TABLE V.—*Results of cultural experiments with certain wood-destroying fungi*

Fungus	Association		Isolation—Pure cultures				Inoculation				Reisolation	
	Symptoms	Hosts	From—	Host	Culture No.	Date	Culture No.	Date	Medium	Date and results	Culture No.	Date and results
Fomes applanatus.	White mottled rot.	Many hardwoods, *Tsuga heterophylla.*	Typical rot.	*Acer saccharum.*	148	Nov. 7, 1921	148	Mar. 17, 1923	*Populus tremuloides;* sapwood+H_2O, heartwood+H_2O.	Oct. 1, 1923; incipient and typical rot, zone lines.	148a	Oct. 18, 1923; positive.
Fomes igniarius.	White spongy rot.	Many hardwoods.	do	*Populus balsamifera.*	154	Nov. 14, 1921	154	Dec. 15, 1922	*Pinus strobus*+H_2O.	May 15, 1923; incipient rot and zone lines.	154a	May 30, 1923; positive.
Do	do	do	Sporophore tissue.	do	154A	Dec. 6, 1921	154A	do	*Populus tremuloides*+H_2O.	Oct. 1, 1923; incipient and typical rot, zone lines.	154b	Oct. 18, 1923; positive.
Fomes laricis	Brown cubical rot.	Several conifers.	Typical rot.	*Pseudotsuga taxifolia.*	268	Mar. 17, 1923	268	Mar. 17, 1923	*Picea sitchensis*+H_2O.	Oct. 1, 1923; incipient and typical rot.	268a	Oct. 20, 1923; positive.
Do	do	do	do	do	268	do	268	do	*Pinus strobus*+H_2O.	do	268b	Oct. 23, 1923; positive.
Fomes pinicola	do	Many conifers and *Alnus tenuifolia.*	do	*Tsuga heterophylla.*	31	Aug. 16, 1920	31	Mar. 14, 1923	*Picea sitchensis*+agar.	Oct. 1, 1923; incipient and typical rot; poroid growths.	31a	Oct. 16, 1923; positive.
Fomes roseus	do	Many conifers.	Sporophore	*Picea canadensis.*	[a] 6364	Dec. 15, 1921	6364	Mar. 10, 1921	Spruce+H_2O	Feb. 1922; typical rot.	6364a	May 7, 1922; positive.
Ganoderma curtisii.	White pocket rot.	*Acer negundo.*	Typical rot	*Acer negundo.*	134	Aug. 29, 1921	134	Apr. 14, 1923	*Pseudotsuga taxifolia*+agar.	Oct. 24, 1923; incipient and typical rot.	134a	Jan. 8, 1924; positive.
Do	do	do	Sporophore.	do	134A	do	134A	do	do	do	134b	Jan. 10, 1924; positive.
Ganoderma tsugae.	do	*Tsuga heterophylla* and *Tsuga canadensis.*	Typical rot	*Tsuga canadensis.*	10F	Nov. 14, 1921	10F	Mar. 14, 1923	*Picea sitchensis*+H_2O.	do	10F	Oct. 31, 1923; positive.
Hymenochaete rubiginosa.	do	Several hardwoods.	do	*Castenea dentata.*	273	Apr. 12, 1923	273	Apr. 14, 1923	*Populus tremuloides.*	Oct. 1, 1923; incipient stage.	273a	Oct. 18, 1923; positive.
Lentinus lepideus.	Brown cubical rot.	Conifers and hardwoods.	Spores	From sporophore *P. taeda.*	[a] 6285	Dec. 15, 1921	6285	Mar. 10, 1921	*Picea sitchensis*+H_2O.	Typical rot and hornlike growths.	6285a	June 6, 1922; positive.
Do	do	do	Typical rot	*P. taeda*	[a] 6285A	do	6285A	Mar. 14, 1923	*Pinus strobus*+H_2O.	Oct. 1, 1923; incipient and typical rot; badly rotted.	6285b	Oct. 17, 1923; positive.

Leucites septaria.	...do...	Many conifers and *Alnus tenuifolia.*	Sporophore.	*Picea canadensis.*	63	Mar. 4, 1921	63	Dec 15, 1921	*Picea sitchensis* +H_2O.	Jan. 6, 1922; typical rot.	63a	Jan. 6, 1923; positive.
Pholiota adiposa.	Brown mottled rot.	*Abies grandis, Tilia americana,* and other hardwoods.	Typical rot.	Sporophore from *Tilia americana.*	243	Jan. 10, 1922	243	Jan. 30, 1922	*Tilia americana* +agar.	Incipient and typical rot; typical sporophores.	248	Dec. 11, 1922; positive.
Do	...do...	...do...	Spores	...do...	18K	Dec. 18, 1921	18K	Jan. 10, 1922	*Tilia americana* +H_2O.	Mar. 3, 1923; incipient and typical rot.	18K	Mar. 15, 1923; positive.
Polyporus adustus (?).	White spongy rot.	*Liquidambar styraciflua.*	Typical rot.	*Liquidambar styraciflua.*	138	Oct. 11, 1921	138	Mar. 17, 1923	*Populus tremuloides;* heartwood+H_2O.	May 15, 1923; typical rot and zone lines.	138a	May 31, 1923 positive.
Do	...do...	...do...	...do...	...do...	138	...do...	138	...do...	*Populus tremuloides;* sapwood+H_2O.	Oct. 1, 1923; incipient and typical rot zone lines.	138b	Oct. 17, 1923 positive.
Polyporus amarus.	Brown pocket rot.	*Libocedrus decurrens.*	...do...	*L. decurrens.*	106	June 29, 1921	106	Dec. 15, 1922	*Picea sitchensis* +H_2O.	May 15, 1923; incipient discoloration; no typical rot.	106a	Oct. 10, 1923; positive.
Polyporus anceps.	White pocket rot.	*Pinus ponderosa* and *Picea canadensis.*	Incipient rot.	*Picea canadensis.*	270	Jan. 30, 1921	270	...do...	*Picea canadensis*+H_2O.	May 15, 1923; typical rot and poroid growths shedding spores.	270a	May 26, 1923 positive.
Do	...do...	*Picea canadensis.*	Typical rot.	...do...	270A	...do...	270A	Mar. 14, 1923	*Pinus strobus* + agar.	Oct. 1, 1923; incipient rot.	270c	Oct. 19, 1923; positive.
Polyporus anceps, 3 tubes.	...do...	...do...	...do...	...do...	270A	...do...	270A	...do...	*Pinus ponderosa;* sap and heartwood +H_2O.	Oct. 1, 1923; incipient rot in heartwood; typical rot in sapwood; poroid growths.	270b	Oct. 15, 1923; positive.
Polyporus balsameus.	Brown cubical rot.	*Abies balsamea* and *Thuja occidentalis.*	...do...	*Abies balsamea.*	230	Oct. 13, 1922	230	Dec. 15, 1922	*Picea canadensis*+H_2O.	May 15, 1923; incipient and typical rot.	230a	May 31, 1923; positive.
Polyporus borealis.	White mottled rot.	*Picea engelmanii, Picea canadensis,* and *Acer saccharum.*	...do...	*Acer saccharum,*	244	Jan. 10, 1922	244	Apr. 14, 1923	*Picea sitchensis* +agar.	Oct. 1, 1923; incipient rot.	244a	Oct. 15, 1923; positive.
Polyporus ellisianus.	White pocket rot.	*Pinus ponderosa.*	...do...	*Pinus ponderosa.*	269	Jan. 30, 1921	269	Dec. 15, 1922	...do...	May 15, 1923; typical rot and poroid growths.	269a	May 31, 1923; positive.
Do	...do...	...do...	...do...	...do...	269	...do...	269	...do...	*Picea sitchensis* +H_2O.	Oct. 1, 1923; incipient and typical rot.	269b	Oct. 12, 1923; positive.

[a] Pure cultures secured from Dr. C. J. Humphrey.

TABLE V.—*Results of cultural experiments with certain wood-destroying fungi*—Continued

Fungus	Association		Isolation—Pure cultures				Inoculation				Reisolation	
	Symptoms	Hosts	From—	Host	Culture No.	Date	Culture No.	Date	Medium	Date and results	Culture No.	Date and results
Polyporus pilotae.	White pocket rot.	*Castanea* and *Quercus* spp.	Typical rot	*Castanea dentata.*	222	July 31, 1922	222	Mar. 14, 1923	*Picea sitchensis* +H_2O.	Oct. 1, 1923; incipient rot.	222a	Oct. 13, 1923; positive.
Polyporus schweinitzii.	Brown cubical rot.	Many conifers.	do	*Larix decidua*	133	Aug. 27, 1921	133	Jan. 9, 1922	*Picea sitchensis* +agar.	Oct. 26, 1922; typical rot.	133a	Oct. 30, 1922; positive.
Do	do	do	do	do	133	Aug. 28, 1921	133	Dec. 15, 1921	*Pinus strobus*, heartwood.	May 15, 1923; incipient rot.	133b	May31, 1923; positive.
Do	do	do	Sporophore tissue.	do	133A	do	133A	do	Spruce+H_2O	Mar. 15, 1923; incipient; Oct. 1, 1923, typical.	133c	Oct. 12, 1923; positive.
Polyporus stipticus.	White pocket rot.	*Pinus ponderosa.*	Incipient rot.	*Pinus ponderosa.*	121	Aug. 17, 1921	121	Apr. 3, 1923	*Picea canadensis*+H_2O.	May 15, 1923; typical rot and poroid growths.	121a	Aug. 6, 1923 positive
Polyporus sulphureus.	Brown cubical rot.	Several conifers and *Quercus* sp.	Typical rot.	Red oak	157	Nov. 17, 1921	157	Dec. 15, 1922	*Picea sitchensis*+H_2O.	May 15, 1923; incipient and typical rot.	157a	July 6, 1923; positive.
Do	do	Conifers and hardwoods.	Incipient rot.	do	157A	do	157A	Mar. 11, 1922	Spruce+H_2O	Nov. 18, 1922; incipient and typical rot.	157b	Dec. 16, 1922; positive.
Polystictus versicolor.	White spongy rot.	Many hardwoods.	Typical rot.	*Ulmus americana.*	255	Feb. 24, 1923	255	Mar. 17, 1923	*Populus tremuloides;* sapwood+H_2O.	May 17, 1923; typical rot.	255a	July 15, 1923; positive.
Pleurotus ostreatus	do	*Ulmus americana*	do	*Populus grandidentata.*	191	Nov. 17, 1921	191	Dec. 15, 1922	*Populus tremuloides* +H_2O	Jan. 6, 1923; typical rot and dimidiate sporophores.	192a	Feb. 12, 1923; positive.
Do	do	*Populus tremuloides.*	Spores	do	192	do	192	do	do	do	192b	Do.
Do	do	*Populus grandidentata* and other hardwoods.	Sporophore tissue.	do	192A	do	192A	do	do	do	192c	Do.
Schizophyllum commune.	White rot	Many hardwoods.	Typical rot and spores.	*Betula papyrifera.*	258	Jan. 10, 1923	258	Mar. 14, 1923	*Pinus strobus* +*agar.*	Oct. 1, 1923; incipient rot and faint typical rot.	258a	Oct. 18, 1923; positive.

Stereum sulcatum.	White pocket rot.	Several conifers.	Typical rot.	*Larix occidentalis.*	33	Aug 25, 1920	33	do	*Pinus strobus* +H_2O.	Jan. 8, 1924; typical rot.	33a	Feb. 16, 1924; positive.
Do	do	do	do	do	33	do	33	do	*Picea sitchensis*+H_2O.	Oct. 1, 1923; incipient rot.	33b	Oct. 31, 1923; positive.
Tramentes carnea.	Brown cubical rot.	Many conifers.	Incipient rot.	*Picea canadensis.*	74	Mar. 18, 1921	74	Dec. 15, 1921	Spruce+H_2O	Oct. 26, 1922; typical rot and poroid growths.	74a	Dec. 20, 1922; positive.
Do	do	do	Sporophore tissue.	do	74A	do	74A	Mar. 14, 1923	*Pinus strobus* +H_2O.	Oct. 1, 1923; incipient and typical rot.	74b	Oct. 15, 1923 positive.
Trametes pini.	White pocket rot.	do	Typical rot.	do	73	Mar. 8, 1921	73	Dec. 15, 1921	Spruce+H_2O	Oct. 26, 1922; typical rot.	73a	Jan. 14, 1923; positive.
Do	do	do	Spores	From sporophores.	[a] 4580	Dec. 15, 1919	4580	Mar. 10, 1921	do	Typical rot	4580a	July 10, 1922; positive.
Trametes serialis.	Brown cubical rot.	Conifers; common *Pseudotsuga taxifolia.*	Typical rot.	*Pseudotsuga taxifolia.*	37	Oct. 19, 1920	37	Dec. 15, 1922	*Picea sitchensis*+H_2O.	Oct. 1, 1923; incipient and typical rot and poroid growths.	37a	Oct. 19, 1923; positive.
Xylaria polymorpha.	White spongy rot, black zone lines.	Maple, basswood, oak.	do	*Acer saccharum.*	146	Jan. 4, 1922	146	Jan. 30, 1922	Basswood + agar.	Nov. 18, 1922; typical rot.	246	Dec. 12, 1922; positive.

[a] Pure cultures secured from Dr. C. J. Humphrey.

DISCUSSION

The methods employed in diagnosing decay in wood have been presented with sufficient detail to show that many of the questions on wood decay asked by both scientist and lumberman can be accurately answered. Proving that decay is present or absent, and that the causal organism is alive or dead, and whether the fungus is a wood destroyer or a staining organism, is relatively easy in a majority of cases. This has been demonstrated by the diagnosis of over 1,500 samples sent to the laboratory for examination during two years. Gross, microsopical, and cultural characters were used in most of the diagnoses. A study of the microsocopical characters is made easier by a collection of microscopical mounts showing the characters of normal sound wood of various species. A similar collection of wood decayed by known fungi can be used to advantage in comparisons made with sections cut from the unidentified sample. A collection of stock pure cultures of the numerous fungi producing decay in wood is indispensible to the successful application of the cultural methods of diagnosis. The way in which cultural characters may furnish the evidence necessary to establish identity of the causal fungus, in addition to macroscopical and microscopical data, is well illustrated by the following examples:

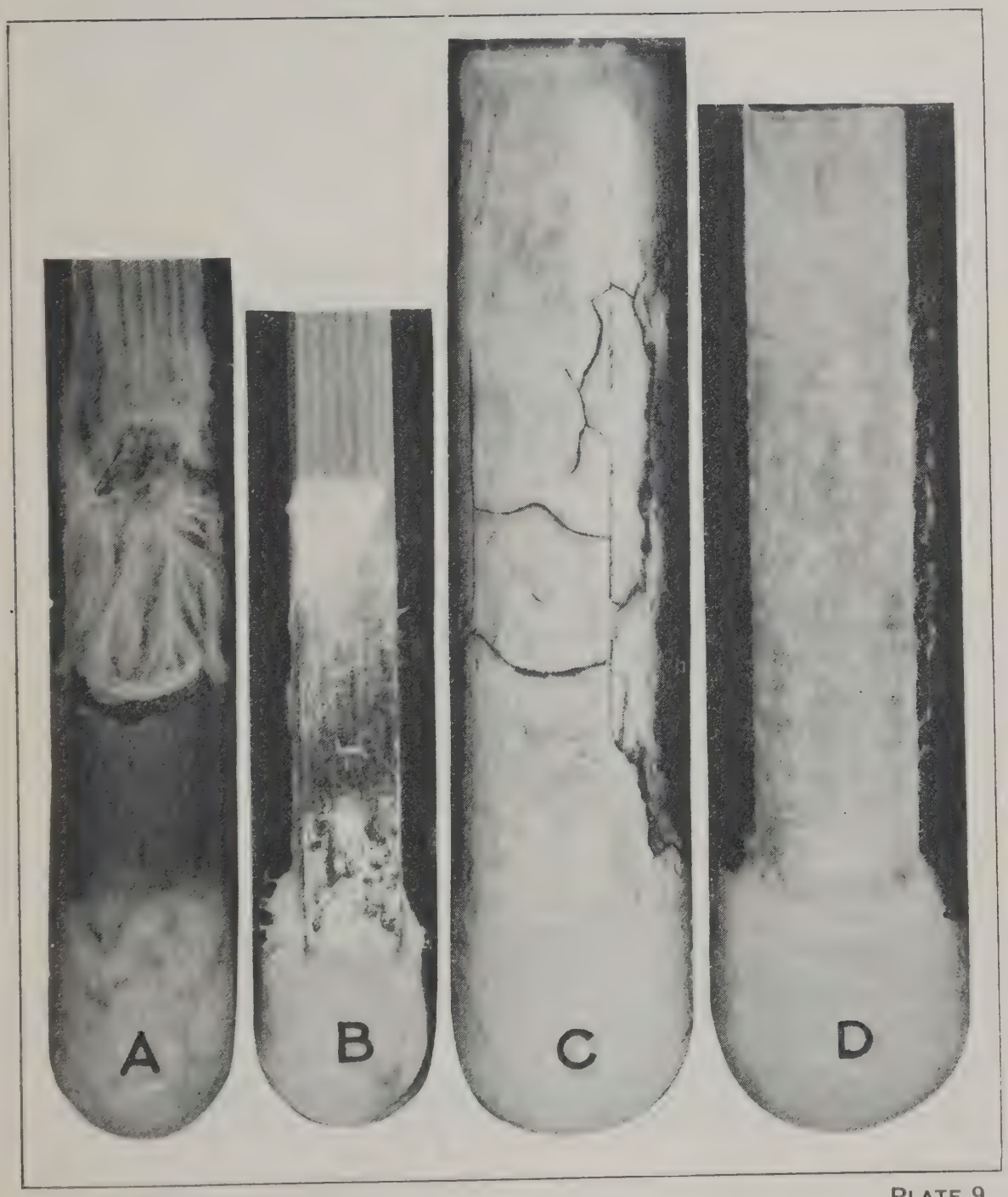
A piece of heartwood oak showing a dark reddish-brown color was submitted for diagnosis. The rot was in the late incipient stage and the gross characters placed it among the brown cubical rots. Microscopical examination showed numerous signs of decay. Clamp connections observed on the hyphae developing in the wood indicated that the fungus was a Hymenomycete. Cultures were made and in a few days mycelium of a bright orange yellow appeared. This was compared with stock pure cultures, and it seemed reasonable to name the causal organism *Polyporus sulphureus*.

A more positive case was a piece of white fir (*Abies concolor*) plank showing a characteristic rot resembling that caused by *Pholiota adiposa*. Cultures on malt agar developed typical though small sporophores of this fungus. In a third case the cultures showed nearly perfect sporophores of *Schizophyllum commune* obtained on malt agar from fragments of infected wood taken from a birch log, and in a fourth case the cultures obtained produced abortive and typical sporophores of *Lentinus lepideus* when fragments of infected railroad ties were used.

The close resemblance of the typical stage of the rots produced by such fungi as *Trametes pini*, *Polyporus anceps*, *Fomes annosus*, *Stereum sulcatum*, *Stereum sanguinolentum*, *Trametes isabellina*, *Fomes nigrolimitatus*, and *Polyporus circinatus* in conifers makes it difficult to determine the causal organism in the absence of sporophores. Cultures aid greatly in this diagnosis and only in the case of *Trametes pini* and *Polyporus circinatus* is there any very close resemblance of cultural characters. In this case careful comparisons will show sufficient differences to distinguish between the two. The cultures of *Polyporus circinatus* on malt agar are a much darker brown than those of *Trametes pini* on the same medium. Other distinguishing characters such as rate of growth, type of surface growth, and discoloration of the medium are to be noted.

Incidentally, the study brings out rather clearly the value of rot characters and cultural evidence in connection with the identification of dubious sporophores of fungi found with the typical rots. The following case is a good example:

Diagnosis was attempted on a section of southern yellow pine fence post with several sporophores of a white polypore attached. The sapwood and part of the heartwood showed large areas of a brown cubical rot. The cultures obtained from the brown rot areas indicated a species of Lenzites (Pl. 10, D), but the sporophores attached showed a white context. *Trametes serialis* was suggested, since it produces a brown cubical rot. However, upon closer examination the early typical stage of a white pocket rot was observed in the sapwood in proximity to the sporophores and merging into the brown rot. The white rot resembled that produced by *Polyporus anceps*. Cultures from the white rot areas, from the sporophore tissue, and from the spores cast by the near-typical sporophore developed on malt agar in the cultures obtained from the brown rot areas furnished sufficient data to identify the sporophore as that of *Polyporus anceps* (Pl. 10, A and B). Sporophores of *Lenzites trabea* were later collected from another part of the same post. These were associated with a brown cubical rot. Cultures of the sporophore tissue were identical with the Lenzites-like cultures obtained as above from the brown rot, and with cultures from the spores cast by the near-typical sporophores developed on malt agar (Pl. 10, D). Therefore in this instance two fungi were isolated and identified from the same sample.



Large test tubes (5 × 40 cm.) containing test sticks inoculated with wood-destroying fungi.

A.—Pure culture of *P. sulphureus* isolated from oak and inoculated on a stick of *Pseudotsuga taxifolia* heartwood with malt agar at the base. 9/16 natural size.

B.—Pure culture of *P. anceps* isolated from *Picea canadensis* and inoculated on a stick of *Picea sitchensis* heartwood moistened with distilled water. 9/16 natural size.

C and D.—Sapwood and heartwood sticks of *Populus tremuloides* inoculated with a pure culture of the fungus (*Polyporus adustus?*) isolated from the orange-zone white rot areas in sap gum lumber. Note the more luxuriant growth in the sapwood stick "C" and the formation of characteristic orange zone lines in the mycelium. 8/9 natural size.

An interesting problem in identity is furnished by the group of white polypores known as *Polyporus anceps*, *Polyporus ellisianus*, and a collection labeled *Polyporus stipticus*. All three of these fungi were associated with white pocket rots in coniferous woods, and the rots resemble each other so closely that minor differences might be ascribed to host relationships. A study of the typical stage of the rots in radial section shows the ends of the pockets usually roughly squared off instead of rounded or pointed. The pockets often contain black flecks. A characteristic incipient discoloration (fig. 2, A) accompanies *Polyporus ellisianus* in *Pinus ponderosa* (*49*) but is less pronounced or at times lacking in *Picea canadensis* and *Abies balsamea* infected with *Polyporus anceps*. This discoloration is prominent in *Pinus banksiana* and *Pinus resinosa* infected with *Polyporus anceps*. *Polyporus anceps*, isolated from *Picea canadensis*, produces on inoculated blocks of *Pinus ponderosa* the characteristic reddish incipient discoloration followed by the typical white pocket rot. The cultures secured from *Pinus ponderosa* infected with *Polyporus ellisianus* or with *Polyporus stipticus* when inoculated on blocks of *Picea canadensis* produced the faint reddish incipient discoloration followed by the typical white pocket rot. A comparison of the pure cultures on malt agar of *Polyporus anceps*, *Polyporus ellisianus*, and the collection labeled *Polyporus stipticus* brings out their close resemblance. A typical character is the formation, always at the upper edge of the slant, of a finely poroid growth which usually has a narrow smooth margin (Pl. 10, A, B). This evidence seems sufficient to warrant identifying as *Polyporus anceps* the three white pocket rots studied in *Pinus ponderosa*, *Picea canadensis*, and *Abies balsamea*.

SUMMARY

Methods for a complete diagnosis of the decays commonly found in wood and wood products are presented, with the study of a representative number of wood-destroying fungi of economic importance.

Decay and its stages in wood are defined and decay processes considered in relation to the study. A classification under the two main groups of white rots and brown rots and their subgroups is presented as a workable grouping according to gross characters.

The methods useful in the diagnosis of decay are given under the three main divisions of gross, microscopical, and cultural characters. These when carefully determined are believed to furnish sufficient data for the identification of the causal organism.

A comparison of the incipient discolorations and zone lines of various wood rots indicates the value of these gross characters in diagnostic tests. The extent to which hyphae penetrate the wood beyond the discolored areas was determined for some fungi by means of microscopical examinations and by cultures. Studies of the methods of cell-wall penetration, size and shape of bore holes, hyphal characters, and general pathological effect upon the cell structures have furnished evidence of the value of microscopical characters, as has also a critical study of the manner of penetration of the hyphae of *Trametes pini* in pine wood.

Cultural characters are found to be of great value in diagnostic studies, in many cases furnishing the evidence necessary to complete the identification of the fungus decomposing the wood.

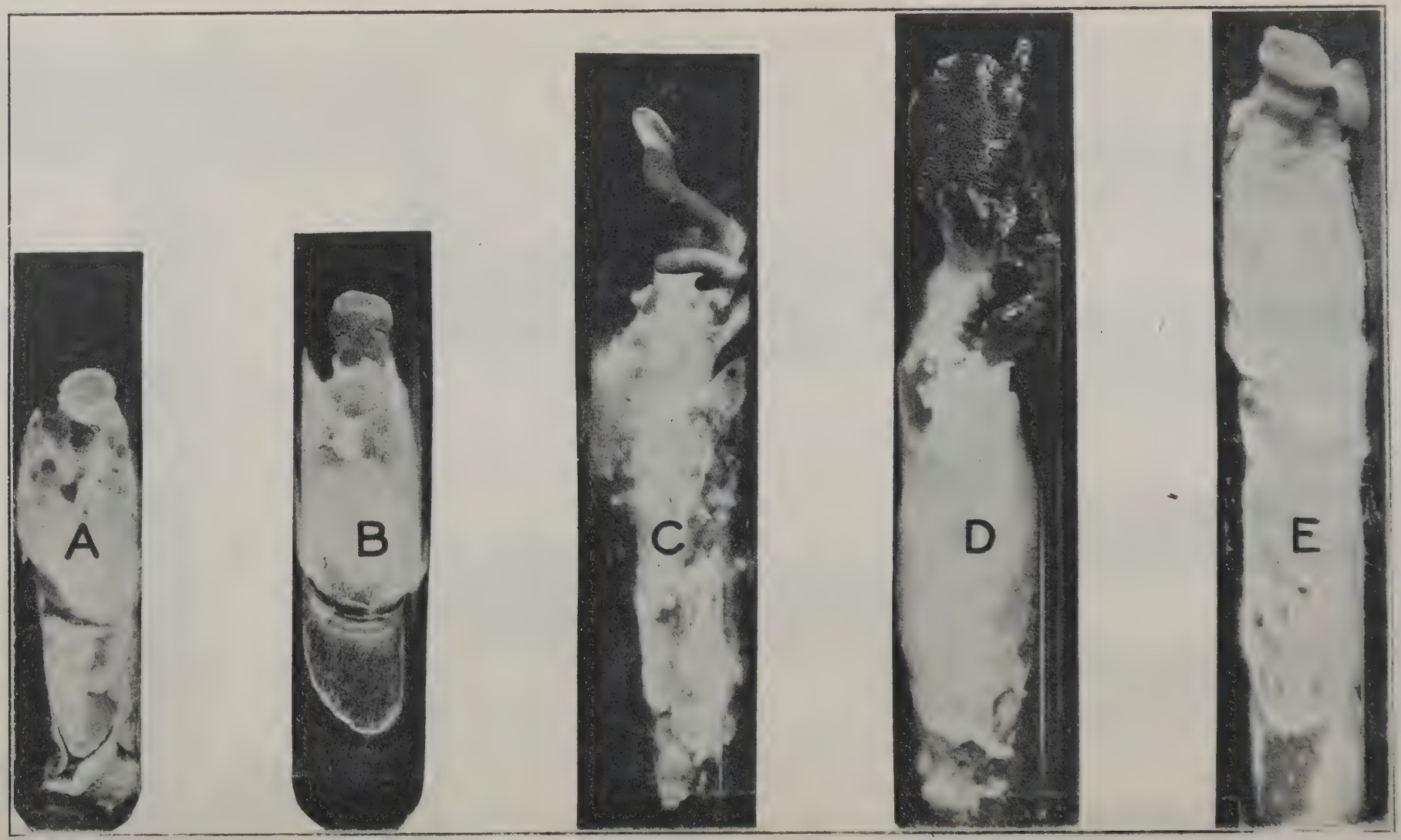
A method is presented which may be used in checking the association of xylophilous fungi with the rots produced. The results of experiments showing this relationship for certain fungi are given.

A complete diagnosis of a sample of wood will determine whether or not decay is present and whether the causal organism is alive or dead, and will furnish data on the nature of the fungus. It will make the detection of decay in otherwise sound wood easier, and in most cases the cumulative evidence obtained will lead to the identification of the fungus causing the rot.

It is believed that use of the diagnostic methods presented will furnish data of scientific value for the solution of numerous problems relating to the staining and rotting of wood and wood products.

EXPLANATORY LEGEND FOR PLATE 10

A and B.—Pure cultures on malt agar of *P. ellisianus* isolated from *Pinus ponderosa* and *P. anceps* from *Picea canadensis*. Note the characteristic poroid growth with smooth margin in both tubes About natural size. C, D, E.—Types of near-typical sporophores produced on artificial media. ×2. C.—*Lentinus lepideus* isolated from *Pinus banksiana* railroad tie. D.—*Lenzites trabea* developing a hymenial layer which cast abundant mature spores. This fungus was isolated from the brown-rot areas in pine post. E.—*Polystictus hirsutus* isolated from birch wood. Identified by typical sporophores attached to the sample.



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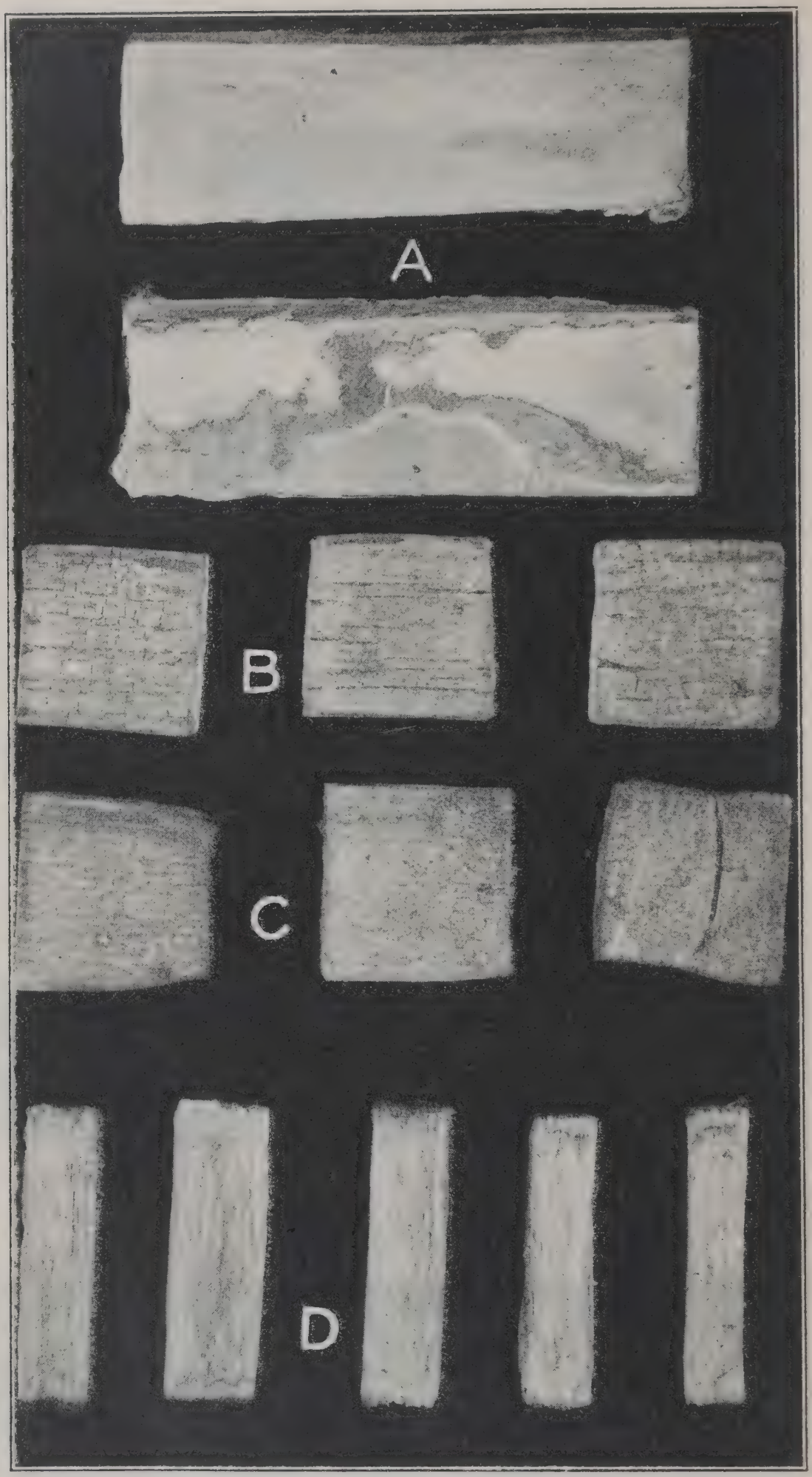

The Diagnosis of Decay in Wood PLATE II

(For explanatory legend see p. 565)

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EXPLANATORY LEGEND FOR PLATE 11

A.—Typical rot and zone line produced in *Tilia americana* by inoculation with a pure culture of *Xylaria polymorpha*. B.—Typical rot produced in *Picea sitchensis* blocks by inoculation with *Lenzites sepiaria*. C.—Incipient and typical rot produced in *Picea sitchensis* blocks by inoculation, with *Polyporus schweinitzii*. At center an almost sound block which had been kept too dry at upper part of tube. The block next to this shows heavy infection and great shrinkage at one end which was adjacent to the moist cotton at bottom of tube. D.—Typical white pocket rot and zone lines produced in *Picea sitchensis* blocks by inoculation with *Trametes pini*. Note the zone lines paralleling the ends of the pieces where evaporation was greatest. Natural size.

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TOTAL ASH DETERMINATION IN SPICES[1]

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The chemical determination most widely used by chemists in passing upon the quality of finely ground spices is that of total ash. This is best obtained by igniting one or two grams of the material, contained in a porcelain or platinum crucible, in a muffle furnace at the lowest temperature that will give a carbon-free residue. Heating for one hour at approximately 700° C. is usually sufficient. Red peppers require a little higher temperature than other spices to obtain the same result. Care must be exercised not to use a higher temperature than a dull red heat, or decomposition of carbonates in the ash will result.

Water-soluble and hydrochloric-acid-insoluble ash are determined usually only when the total ash or microscopic examination arouses suspicions of impurity or adulteration. If the total ash is low for the spice under consideration and the water-soluble ash very low the spice has probably been extracted. If the reverse is true of the total and acid-insoluble ash the spice contains extraneous mineral matter.

The color and appearance of the ash often tell something about the spice from which it was derived. Pure red peppers give a light greenish-blue ash, which is due to the presence of copper. American saffron or safflower has a red-brown ash; Spanish saffron yields a white or very pale gray ash; cinnamon ash is white or nearly so, and that of cassia is brown or brownish gray; clove ash is dark green, while most other kinds are white or gray. If spice ash is rubbed between the fingers the presence of foreign mineral matter in the original will be evidenced by a gritty feeling.

In spite of the simplicity of a total ash determination, the figures reported by different investigators vary considerably. A survey of the literature reveals the following partial list of figures as the average percentage of total ash in pure ginger: 4.39, 5.36, 5.55, 6.01, 3.80, 5.27, 3.62, 6.78, 3.66, 5.88, and 4.46. The first four are averages of more than 50 determinations each. Similar variations occur in results reported for other spices. This is chiefly due to variation in the spice itself and to a less extent to the personal equation.

Richardson (*89*) [2] gives the total ash in Acheen black pepper as 8.99 per cent and that of Singapore black pepper as 5.41 per cent. These peppers are obtained from the same botanical species and the difference is largely due to variations in the circumstances surrounding their production, such as soil, climate, and handling.

Almost as large differences occur in spices from the same source from year to year as in those from different sources. This is due to differences in climatic conditions and has been well shown by Sindall (*11*). Table I has been prepared from some of his figures, each of which is the average of a large number of determinations upon pure cinnamon, imported from the same sources in successive years.

TABLE I.—*Per cent of ash in cinnamon*

	1908	1909	1910	1911
China cinnamon	4.79	3.77	3.84	3.27
Batavia cinnamon	4.68	3.79	3.78	4.32

In 1908 the average ash content of all samples from China was higher and in 1911 lower than that of Batavia samples in any year. The averages of the best figures available for four different varieties of cinnamon come between 4 and 4.15 per cent.

This variation is further shown by the percentages following which are the averages presented by numerous investigators for different varieties of black pepper.

[1] Received for publication May 22, 1924—issued February, 1925.

[2] Reference is made by number (italic) to "Bibliography," pp. 572–574. Citations in the bibliography which are not referred to in the text were used in the preparation of the tables.

TABLE II.—*Per cent of ash in different varieties of black pepper*

Batavia	4.93	10.90					
Acheen	8.99	5.14	5.17	4.56	6.44		
Trang	8.85	4.21	4.66				
Singapore	3.48	3.36	5.93	3.20	4.42	5.41	5.39
Tellicherry	5.28	6.41	4.17	4.38	5.35	4.43	
Penang	4.62	6.44	4.02	5.67	4.59	4.74	
Allepo	3.27	4.59	3.85	4.43			

TABLE III.—*Total ash determinations of various spices*

Air-dried spice	Number of samples	Number of analysts	Representative total ash	Total ash permissible under pure food laws (*110*)
			Per cent	*Per cent*
Allspice	296	15	4.51	6
Anise	32	6	6.85	9
Bay leaves	29	5	4.63	
Caraway	51	7	6.73	8
Cardamom fruit	109	12	6.71	8
Cassia	143	4	4.28	5
Celery seed	101	4	8.35	10
Cinnamon	542	19	4.05	5
Cloves	451	13	6.15	7
Coriander	270	4	5.38	7
Cumin	14	4	7.63	8.5
Fennel	48	7	8.12	9
Ginger	841	20	4.89	7
Mace	219	10	2.26	3
Marjoram	156	5	10.62	16
Mustard	151	21	4.83	5
Mustard flour	26	4	5.49	6
Nutmeg	342	8	2.45	5
Onion	14	5	4.28	
Paprika	449	12	6.78	8
Pepper, black	581	23	5.03	7
Do.... cayenne	237	15	6.17	7
Do.... other red varieties	159	4	6.47	8
Do.... white	660	13	1.26	3.5
Safflower	18	4	6.67	
Saffron	156	14	5.38	
Sage	311	2	7.39	10
Savory	29	4	9.94	
Star anise	63	3	2.63	
Thyme	104	4	9.83	14
Turmeric	36	9	6.37	

Similar results have been reported for other spices. It seems useless, therefore, to consider the geographical source of a spice in interpreting a total ash determination except where a consistent difference is well authenticated. Sage seems to be one of the few such spices, for the American-raised variety appears to show consistently a higher percentage of ash than the Austrian.

While numerous figures are available for the percentages of ash in the commoner spices, few reliable results are published for the spices principally used by manufacturers, such as dill, turmeric, fennel, etc.

In order to draw conclusions from a total ash determination, it is desirable to know what percentage should be present when all factors tending to vary it have been eliminated. There are no such figures available at the present time. Each text book and article giving results of ash determinations reports a different figure for the same spice. This is not at all surprising when we consider that most of these figures are averages obtained with a few samples purchased at the same time and in the same market. When we remember further that different degrees of heating will result in different weights of ash from the same sample we understand why some chemists report twice as much ash as others for the same spice.

The percentages for total ash given in Table III are the result of an effort to find the amount of ash in the spices listed when all the conditions affecting

it had been averaged. A list was first made of every available average obtained with goods known to be pure, for each kind of spice. Such figures were obtained from most of the articles listed in the bibliography. A few results which were clearly unreliable were then eliminated, and weighted averages prepared from those remaining. The determinations upon which they are based were made by many different analysts over a period of forty years. Many of them have not previously been published. The unpublished determinations, numbering several thousand, were made chiefly by James Blaine Martin, for the use of whose results grateful acknowledgment is hereby made, and by the author, in the Meat Inspection Laboratory of the United States Department of Agriculture. All the samples were examined physically and microscopically, and no determination on questionable material was used in the preparation of this table.

Variations from these figures should not exceed one-third of their value.

For comparison there is included in this list the maximum total ash permissible by the Bureau of Chemistry in the enforcement of the pure food laws.

The approximate ash content of other spices used to some extent in the powdered form is given in Table IV. It is not claimed that these figures are representative, for they are averages of only a few determinations in each case. However, inasmuch as there are no others available, they are given for whatever value they may have.

The approximate composition of the ash derived from several spices is shown by the composite analyses tabulated below.

TABLE IV.—*Approximate ash content of spices named*

	Per cent
Basil	15.12
Calamus	4.64
Capers	2.11
Cassia buds	4.71
Charlock	6.15
Dill	9.88
Fenugreek	3.01
Garlic	6.43
Juniper berries	2.71
Parsley	11.70
Tarrican	10.43
Vanilla beans	4.78

TABLE V.—*Approximate composition of ash from spices named*

	Black pepper	White pepper	Mustard	Paprika	Cinnamon	Cassia	Cardamon	Marjoram	Fenugreek
K_2O	27.56	6.13	18.90	54.37	14.23	5.55	10.32	19.22	33.20
Na_2O	3.89	.79	.37	3.98	4.02	.91	20.01	.67	5.51
CaO	13.73	32.07	15.57	5.15	39.02	51.30	13.20	20.05	8.57
MgO	7.55	10.58	10.51	6.02	3.35	1.19	4.56	5.67	7.10
Fe_2O_3	.58	2.04	1.09	1.97	.48	6.11	.32	6.68	2.33
Al_2O_3				.09			1.57		
Mn_3O_4	.20	.55			.75	1.18	5.05	.65	.10
CuO				.10					
P_2O_5	9.42	29.54	38.22	16.43	2.97	1.15	6.00	8.98	15.01
SO_3	8.48	3.14	5.76	5.70	2.68	.64	11.74	4.86	7.89
Cl	9.13		.17	3.51	.56	.11	2.35	1.76	4.97
CO_2	12.90	14.81	2.62		31.55	31.02	4.51	7.26	10.66
SiO_2	6.56	.35	6.79	2.68	.39	.84	20.37	24.20	4.66

SUMMARY

1. The ash content of spices is affected by numerous factors, so that analyses made upon samples derived from a particular source and crop are not likely to be representative for that spice in general.

2. Figures representing the percentage of total ash which should normally occur in practically all spices in general use have been presented.

3. Composite analyses of the ash of several spices are given.

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LIFE-HISTORY STUDIES OF THE TOBACCO FLEA-BEETLE IN THE SOUTHERN CIGAR-WRAPPER DISTRICT [1]

By F. S. CHAMBERLIN, *Assistant Entomologist*, and J. N. TENHET, *Junior Entomologist, Southern Field Crop Insect Investigations, Bureau of Entomology; technical description of the larva by* ADAM G. BÖVING, *Entomologist, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The tobacco flea-beetle [2] has been recognized for many years as a serious tobacco pest, especially in the tropical and semitropical tobacco-growing regions. Experiments and observations bearing on the control of this pest were carried on by the Bureau of Entomology at Quincy, Fla., from 1918 to 1923 (*2*).[3] In connection with this work a detailed life-history study in the southern cigar-wrapper district [4] was undertaken in 1920 and continued through 1923.

While a number of writers have discussed the life history of this insect, especially Chittenden (*3*, *4*), Howard (*5*), Morgan (*9*), and Metcalf (*7*, *8*), until the present time no detailed life-history records have been published.

REARING METHODS

Various means for obtaining eggs were tested during the course of this investigation. The method employed by Johannsen (*6*) in obtaining eggs of the potato flea-beetle was found to be the most satisfactory. The beetles are enclosed in a lantern globe, which is covered at each end with organdy and set in a vertical position over a flowerpot. The globe is placed upon a piece of black blotting paper kept damp by contact with moist earth in the flowerpot. The beetles thrust their ovipositors through the cloth and deposit their eggs on the paper, where they can be easily seen. In using this method it was found that a certain number of eggs invariably adhered to the meshes of the cloth. A count of the eggs was most easily obtained by using a dark-colored organdy on the end of the globe in contact with the paper.

To obtain incubation records, the deposition cages were placed on end in direct contact with firmed, moist soil contained in tin salve boxes, and the eggs were deposited directly on the surface of the soil. The larvae were reared in these boxes of earth over which large salve boxes were inverted. Food was supplied the larvae by placing sprouted tobacco seed on the surface of the earth. The moisture content of the earth was kept as uniform as possible by the occasional addition of a few drops of water.

Pupal records were most easily obtained by placing a piece of dark blotting paper over the earth in the boxes about the time that the larvae reached maturity. The pupal cells were formed in the earth directly beneath the blotting paper, where they could be easily observed. Although this work was performed on the laboratory porch, it was found that the soil temperature under these artificial conditions varied but slightly from the temperature of the soil beneath tobacco plants in the field, where the tobacco flea-beetle larvae and pupae normally exist.

DESCRIPTION OF STAGES

THE EGG

The egg (fig. 1) is elongate oval, slightly more pointed at one end, 0.362 to 0.483 mm. in length and 0.164 to 0.259 mm. in diameter, translucent and pearly white, gradually assuming a faint lemon-colored tinge as it grows older. Under the compound microscope the chorion apparently shows a slight but distinct reticulation.

THE LARVA

MATURE LARVA.—Larva delicate, threadlike, measuring about 3 mm. Color white, except on the chitinized parts, which are light brown; head capsule with dark margins and end of ninth abdominal segment blackish brown. Head small,

[1] Received for publication April 22, 1924—issued March, 1925.
[2] *Epitrix parvula* Fab.; order Coleopetra, family Chrysomelidae.
[3] Reference is made by number (italic) to "Literature cited," p. 584.
[4] Northern Florida and southern Georgia.

somewhat elongate and nutant. Labrum anteriorly rounded, with a row of short setae, two on each side. Clypeus transverse, also with a single row of small setae, three on each side. Frons distinct from epicranium; several short setae both on frons and epicranium. Ocelli absent. Antenna (fig. 3, B) short, attached to cranium by a large membrane (*m*), two-jointed; basal joint ring-shaped with two setae; apical joint minute, anteriorly with two horn-shaped prolongations (*h*); supplementary appendix (*a*) conspicuous, ovate. Mandible (fig. 3, D) ending with four smooth teeth, the two median ones falciform and larger than the two others; the proximal of the latter somewhat enlarged at base, the distal one slender and pointed; three long setae at inner margin near base and one long and strong seta on the external side of the mandible. Maxilla (fig. 3, C) with large and broad lobe (*l*), which on the inner margin carries a series of rather strong, stiff setae and at the end several smaller hairs in a ring around a papilla (*p*). Maxillary palpus conical, three-jointed, with terminal joint longer than the two others and medianly on the

FIG. 1.—*Epitrix parvula:* Eggs. Greatly enlarged

outer side carrying a single strong seta. Labium proper (*e*, fig. 3, C) transverse, posteriorly limited by a thinly chitinized arch; one short seta on each side. Labial palpus short, two-jointed. Ligula lacking. Mentum and submentum (*mt*) not separated, together forming a large, membranous, rounded region between the maxillae, with an anterior and posterior pair of setae. Prothorax with slightly chitinized tergal shield, the two other thoracic segments entirely fleshy. All thoracic segments sparsely provided with setae.

Legs (fig. 3, A) short and rather weak; all equal and five-jointed; the fifth joint or "claw" hook-shaped, very curved and pointed; large membranous empodium (*e*) present.

First to eighth abdominal segments (fig. 3, F, H) cylindrical and separated by large intersegmental membranes (*i*). Dorsally each segment is divided into three folds, the anterior (*1*) carrying three short setae on each side, the second (*2*) one on each side, and the third (*3*) two on each side. Behind the spiracle (*s*) a single seta (*4*), placed in direct continuation of the two belonging to the third fold. Epipleural (*e*) and hypopleural (*h*) areas present, separated by distinct ventrolateral suture, and each carrying two short setae. Ventrally the segment has two transverse short folds (*v*, *w*) each with a few setae. At the base of all the setae are very inconspicuous, smooth and shiny, rounded, thinly chitinized plates.

Ninth abdominal segment (fig. 3, G), spatulate, posteriorly rounded; two pairs of long setae on its dorsal surface, the one pair (*1*) anteriorly, the second pair (*2*) posteriorly placed; laterally and posteriorly with three shorter but well developed setae on each side.

Tenth abdominal segment (fig. 3, E) retractile, cylindrical, and long, with the character of a locomotory organ.

Spiracles ring-shaped, small; one mesothoracic pair and eight abdominal pairs present.

LARVAL INSTARS.—First-instar larva 0.7 to 1.4 mm. long; white except the head, which is yellowish in color. Second-instar larva about 2 mm. long; white in color, with digestive tract showing darker. Last-instar larva about 3 mm. long; color white except on the chitinized parts, as the head capsule, which are light brownish.

THE PUPA

Uniformly white when first transformed. Length about 1.670 mm., width across mesothoracic femur about 0.959 mm. General appearance characteristic of the Chrysomelidae. Head bent downwards. Antennae directed caudad, about four of middle segments concealed beneath the prothoracic and mesothoracic legs, last three segments directed toward mesal line and lying posterior and nearly parallel to the tarsi of the mesothoracic legs. Metathoracic legs only partially concealed beneath elytra and wings, femurtibial elbow approximately even with tip of elytra. Elytra overlapping fourth abdominal segment, wing almost attaining sixth segment of abdomen. Caudal segment of abdomen bearing a pair of characteristic hooklike appendages.

THE ADULT

The following description of *Epitrix parvula* is by W. S. Blatchley (*1*).

Oblong-oval, subconvex. Dull reddish-yellow elytra often with a fuscous transverse cloud at middle; abdomen brown; antennae and legs pale reddish-yellow, the four outer joints of former and hind femora of latter often darker.

Thorax convex, shining, nearly twice as wide as long, not narrowed in front; antebasal impression evident but not deep; surface distinctly but rather finely and sparsely punctate. Elytra very little wider than thorax, umbone feeble, punctures rather coarse and not crowded on disk: finer and more close-set on sides, where the intervals are subconvex. Length 1.5 to 2 mm.

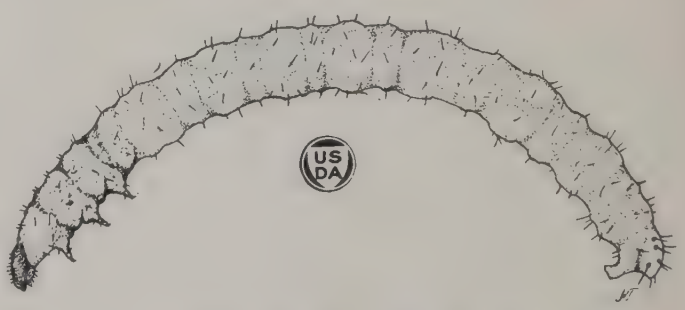

FIG. 2.—*Epitrix parvula*: Full-grown larva. × 17

LIFE HISTORY AND HABITS

LIFE-HISTORY SUMMARY

The eggs of the beetle are deposited in the soil near the base of the tobacco plant. They hatch in a few days and the larvae begin to feed on the small rootlets. The larval stage, which includes three instars, requires about 29 days at Quincy, Fla., during the cool weather of early spring, but in the hot weather of midsummer this period may be shortened by more than one-half. The pupal stage is passed in a small oval-shaped cell just below the surface of the soil. Newly emerged adults confine their feeding to the lower leaves

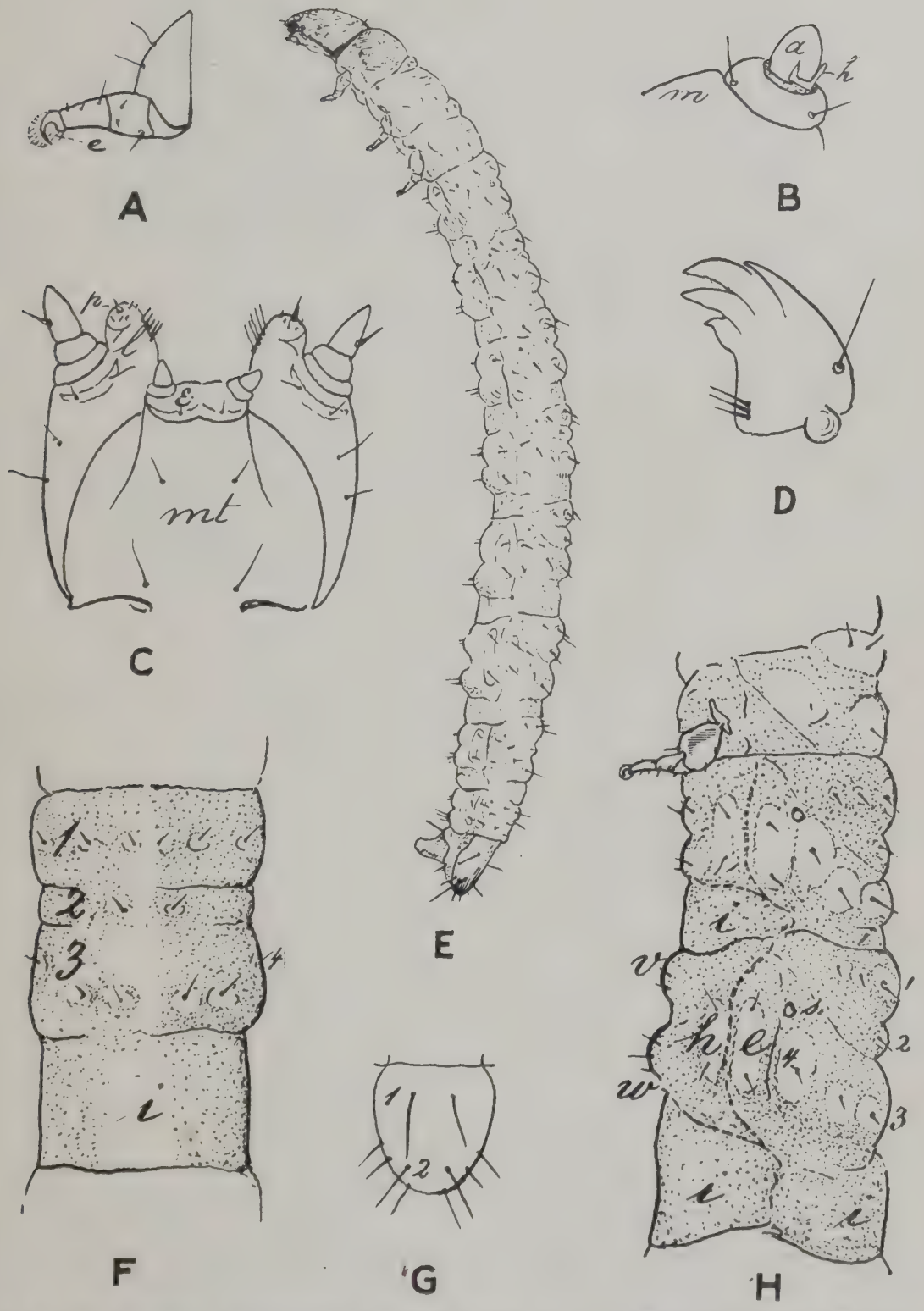

FIG. 3.—*Epitrix parvula:* Morphological details of mature larva. A, First thoracic leg: *e*, Empodium. B, Antenna: *m*, Basal membrane; *h*, apical joint; *a*, supplementary appendix. C, Ventral mouth parts *mt*, Mentum (=submentum); *e*, eulabium; *l*, lacinia; *p*, papilla. D, Left mandible, ventral view. E, Mature larva, lateral view. F, Second abdominal segment, dorsal view: *1*, First fold; *2*, second fold; *3*, third fold; *i*, intersegmental membrane. G, Ninth abdominal segment, dorsal view: *1*, Anterior long seta; *2*, posterior long seta. H, Third thoracic and first and second abdominal segments, lateral view: *i*, Intersegmental membrane; *s*, spiracle; *e*, epipleurum; *h*, hypopleurum; *w*, anterior ventral fold; *1*, anterior dorsal fold with three setae; *2*, median dorsal fold with one seta; *3*, posterior dorsal fold with two setae; *4*, single seta behind spiracle

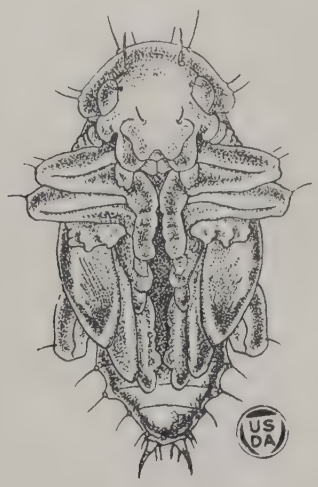

FIG. 4.—*Epitrix parvula:* Pupa. × 11

FIG. 5.—*Epitrix parvula:* Adult. × 20

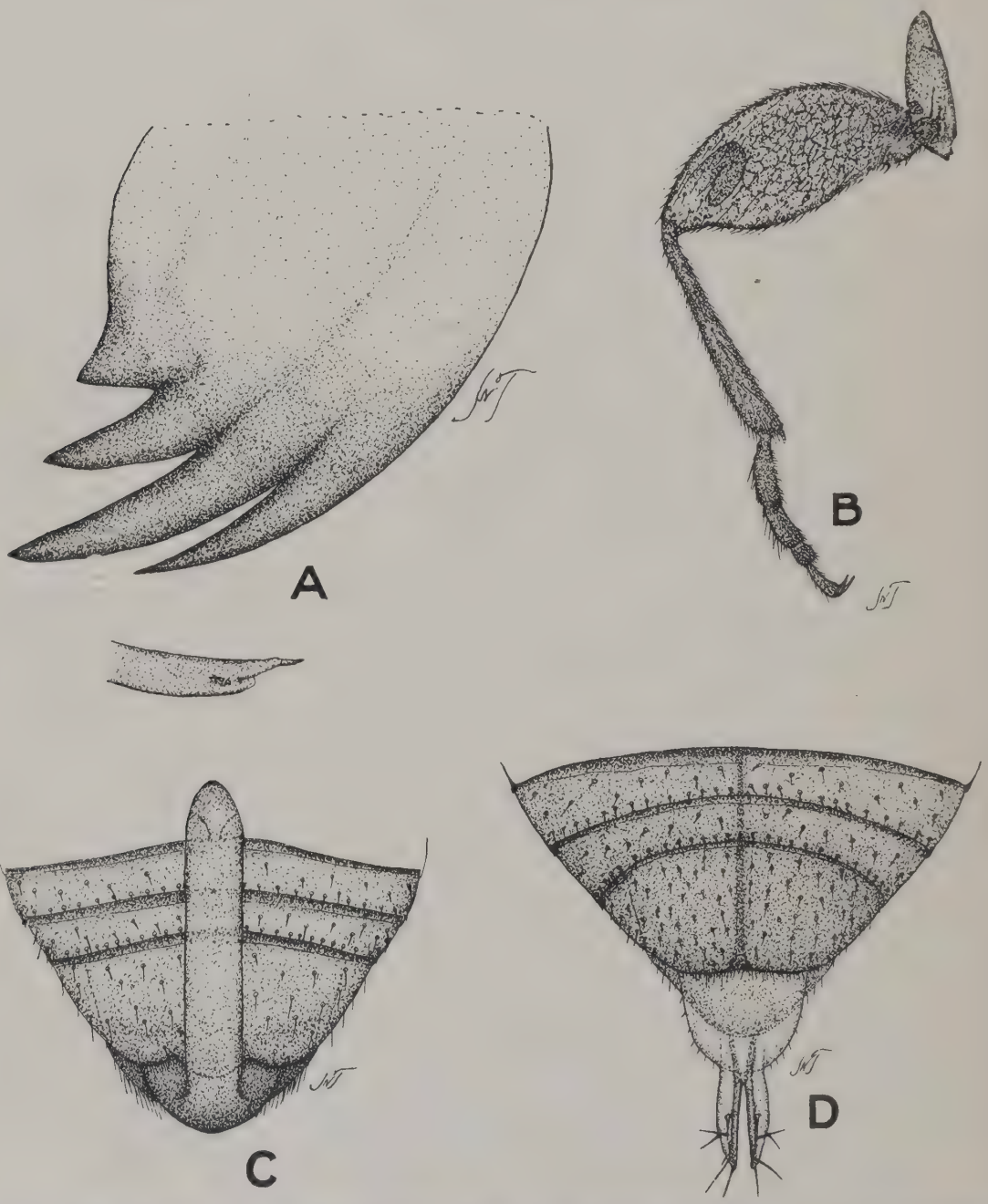

FIG. 6.—*Epitrigparvula:* A, Mandible of beetle. ×250; B, rear leg of beetle. ×58; C, tip of abdomen of male beetle showing genitalia extruded, with lateral view of tip of penis above. ×58; D, tip of abdomen of female beetle, showing ovipositor partially extruded, ventral view. ×58

which touch the ground, but older beetles feed on all portions of the foliage.

The tobacco crop is attacked by the overwintered generation and by two later generations of flea-beetles. After the crop is harvested there may be two and possibly three additional generations, which so overlap that it is impossible to tell them apart.

The winter is passed in the adult stage in a more or less incomplete state of hibernation.

THE EGG

HOW AND WHERE DEPOSITED.—The egg is deposited on or near the surface of the soil in cracks and crevices; usually with the point downward so that only the top of the larger end can be seen. It may be deposited, however, in almost any position; on end, lying on its side, or at an angle, depending more or less on the nature of the crevice in which it is placed. When tobacco is set out in the fields the eggs are usually laid in the moist depression about the base of the stalks where the plant is watered at setting time. Later in the season, when the plants are larger, most of the eggs seem to be deposited some little distance away from the stalk, beneath the lower leaves resting on the ground, where the beetles find more moisture and protection.

NUMBER OF EGGS DEPOSITED.—The number of eggs deposited by a single female appears to vary greatly; this variation doubtless is due to many factors. Under cage conditions, one lot of overwintered females confined with males laid an average of 164.47 eggs. Since the average longevity of the females in this cage was 44.8 days, an average of 3.67 eggs was deposited daily by each female. In this experiment, however, the beetles were not collected until the middle of April, and since oviposition in this locality frequently commences at a much earlier date, it is probable that a number of eggs were deposited before the flea-beetles were obtained. It can, therefore, be assumed that at least 200 eggs may be deposited by a female of this generation.

Two similar experiments with adults of the spring generation, in which a total of 9,257 eggs was deposited, gave an average deposition per female of 100.73 eggs. In both of these experiments the beetles used were collected within two or three days after emergence, so that these records may be taken as fairly indicative of the number of eggs deposited by the spring generation.

All records so far obtained indicate that the overwintered females deposit a much larger number of eggs than the females of the later generations. If this is correct, it is perhaps a provision of nature to counterbalance the heavy winter mortality.

Very few deposition records of individual females have been obtained by the authors. In one case, however, a single female deposited 12 eggs within 24 hours. Other records have been obtained of as high as 21 eggs deposited in a mass, more or less adhering together, all apparently having been deposited by one female.

TIME OF DAY WHEN OVIPOSITION OCCURS.—Eggs may be deposited at almost any hour of the day or night. The summarized deposition records shown in Table I indicate that the flea-beetles have no special time at which they deposit their eggs. In obtaining these records, which cover a period of 11 days, the deposition cage was placed throughout the day on the ground under a tobacco plant in the field, where a nearly normal condition of sunlight prevailed. During the night the cage was kept on the laboratory porch.

INFLUENCE OF SOIL MOISTURE ON DEPOSITION OF EGGS.—Soil moisture appears to have a considerable influence upon the deposition of eggs. Moist soil, when available, is always selected in preference to dry soil. In one case 350 flea-beetles, confined in a lantern-globe cage, were given an equal choice of either moist or air-dry soil upon which to oviposit. Over a 60-day period only 9 eggs were deposited on the dry soil as against 3,146 eggs deposited on the moist soil.

Where moist soil is unavailable upon which to oviposit the beetles deposit eggs very sparingly, even over long periods of time. Two deposition cages, each containing 150 flea-beetles, were started at the same time, the first being supplied with air-dry soil, the other with moist soil. Over a 60-day period 287 eggs were deposited on the dry soil as against 5,324 eggs deposited on the moist soil.

INFLUENCE OF TEMPERATURE UPON EGG DEPOSITION.—Deposition records of the tobacco flea-beetle indicate that no eggs are deposited when the daily mean temperature is below 50° F. No relationship between the number of eggs deposited and prevailing higher temperatures could be observed.

INFLUENCE OF TEMPERATURE ON LENGTH OF INCUBATION PERIOD.—Temperature seems to be the deciding factor in regulating the length of the incubation period. In the early spring

months at Quincy, Fla., incubation requires about 11 days, whereas in summer the eggs usually hatch in about 5 days. The period may, however, vary considerably within the same range of temperatures. Eggs deposited the same day sometimes vary as much as 4 days in the date of hatching.

TABLE I.—*Summarized deposition records of the tobacco flea-beetle obtained June 1 and 2, 1923, at Quincy, Fla., showing time of day at which eggs are deposited*

Time interval	Eggs deposited	Percentage
5 a. m. to 7 a. m.	130	9.0
7 a. m. to 12 m.	211	14.5
12 m. to 6.30 p. m.	366	25.2
6.30 p. m. to 8 p. m.	208	14.3
8 p. m. to 10.30 p. m.	244	16.8
10.30 p. m. to 5 a. m.	294	20.2
Total	1,453	100.0

other hand, gave as high a per cent hatch as in the case of soils of medium moisture content.

The greatest difficulty in life-history studies was encountered in the larval stage, owing primarily to the fact that the moisture requirements for this stage are so exacting. A certain amount of soil moisture is absolutely essential for larval development, but excessive moisture may prove to be very detrimental. On the other hand, the larvae seem to be able to develop successfully under a considerable range of temperature.

When maturity is reached the larva forms a small ovoid-shaped cell about an inch below the soil surface, and begins to shorten preparatory to pupation.

THE LARVA

DESCRIPTION OF LARVA EMERGING FROM EGG.—An egg under observation beneath the binocular microscope began hatching at 12.41 p. m. The larger end of the egg burst open as though

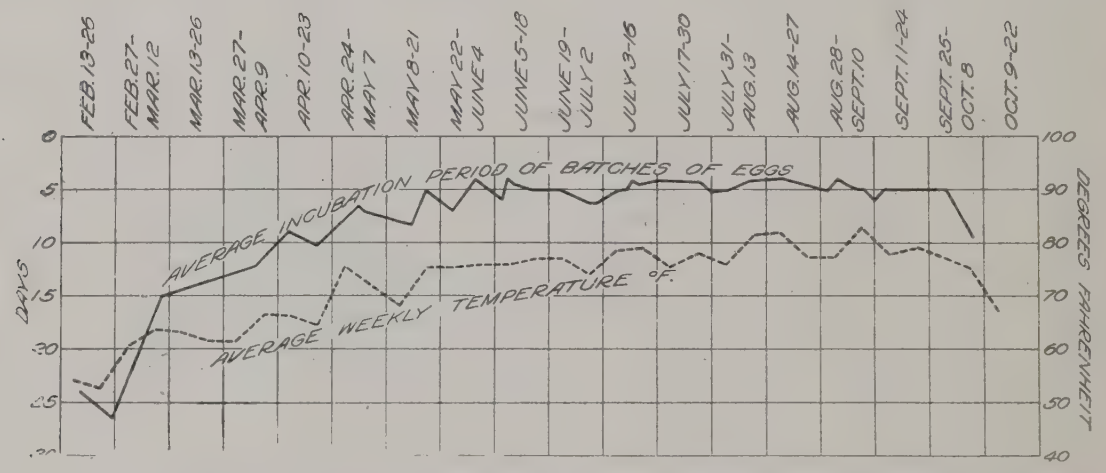

FIG. 7.—Relation of temperature to incubation of eggs of *Epitrix parvula*, Quincy, Fla., 1923

This effect of temperature upon the incubation of the tobacco flea-beetle egg is very marked. In one case, eggs deposited on February 12 required 26 to 27 days for hatching, whereas several records giving an incubation period as short as 3 days have been obtained in midsummer. The relationship between mean temperature and the length of the egg period is shown in Figure 7.

INFLUENCE OF MOISTURE ON INCUBATION.—The eggs of *E. parvula* require a certain amount of moisture for hatching, and the incubation period probably varies to a certain extent with the moisture conditions. Eggs placed by the beetles upon air-dry soil all failed to hatch, and eggs deposited on only slightly moistened soil gave a comparatively low hatch. Eggs deposited on water-saturated soil, on the

the larva had gnawed through from the inside. The head shield of the larva could be plainly seen. The head moved rapidly in a rotary manner, enlarging the opening, and was soon thrust out of the shell. After the head was free, movement ceased, except for a slow rhythmic movement of the body which could be plainly seen through the shell, and the larva rested for nearly a minute. Then the movement of the body quickened, the head moved rapidly from side to side, and in the course of about two minutes of unceasing exertion all of the prolegs were liberated. As soon as each proleg was freed it was brought into play. The larva twisted and pulled almost continuously, pausing for a second now and then, only to begin again with renewed efforts. At the end of exactly eight and one-half minutes from the

time the egg was ruptured, the anal segment was pulled clear of the shell, and the tiny larva crawled under the shelter of a large grain of sand to rest.

HABITS AND BEHAVIOR.—When first hatched, the tiny whitish larvae quickly start an active search for food. In their normal subterranean habitat they probably work their way through the loose soil, but in the covered rearing boxes of firmly packed earth, when food was available, they seldom burrowed below the surface.

The natural food of the tobacco flea-beetle larva is the tiny fibrous rootlets of the tobacco plant and other plants of the family Solanaceae. The young larva frequently bores into a root and tunnels it, but this habit, though common in the first instar, seems rare in the second and third instars. The older larva usually girdles a root or gnaws completely through it, severing it from the plant. No records of larvae feeding above ground have ever been obtained except under laboratory conditions. In the laboratory the best results were obtained by feeding with sprouted tobacco seed. Under these conditions several larvae have been observed to feed upon the small green cotyledonous leaves. Once the larva has begun feeding, it seldom moves about except in search of more food.

TABLE II.—*Length of instars of the tobacco flea-beetle (Epitrix parvula Fab.) at Quincy, Fla., 1922*

Date and time of day egg hatched	Date and time of day larva molted to second instar	Length of first instar	Date and time of day larva molted to third instar	Length of second instar	Date and time of day larva pupated	Length of third instar	Total length of larval stage
1922	1922	*Days*	1922	*Days*	1922	*Days*	*Days*
May 12, a. m.	May 20, a. m.	8	May 25, p. m.	5½	June 3, a. m.	8½	22
Do.	May 19, a. m.	7	May 22, m.	3¼	May 29, p. m.	7¼	17½
Do.	May 18, a. m.	6	May 21, m.	3¼	May 30, a. m.	8¾	18
May 12, m.	May 19, a. m.	6¾	May 23, a. m.	4	June 1, m.	9¼	20
May 13, a. m.	May 20, a. m.	7	May 24, a. m.	4	June 4, a. m.	11	22
Do.	May 19, a. m.	6	May 23, a. m.	4	May 30, p. m.	7½	17½
May 22, p. m.	May 27, p. m.	5	May 31, p. m.	4	June 8, a, m.	7½	16½
May 23, m.	May 28, m.	5	June 2, p. m.	5¼	June 9, a. m.	6½	16¾
Do.	May 28, a. m.	4¾	June 1, p. m.	4½	June 8, m.	6¾	16
Do.	May 28, p. m.	5¼	June 2, p. m.	5	June 8, a.. m.	5½	15¾
Do.	May 27, p. m.	4¼	June 1, p. m.	5	June 7, p. m.	6	15¼
Do.	May 28, a. m.	4¾	do	4½	June 8, m.	6¾	16
Do.	May 28, p. m.	5¼	June 2, a. m.	4½	June 8, a. m.	6	15¾
Do.	May 27, p. m.	4¼	May 31, a. m.	3½	June 10, a. m.	10	17¾
Do.	do	4¼	May 31, p. m.	4	June 7, a. m.	6½	14¾
Do.	May 28, a. m.	4¾	June 1, p. m.	4½	June 9, a. m.	7½	16¾
Do.	May 27, a. m.	3¾	May 30, p. m.	3½	June 7, a. m.	7½	14¾
Do.	May 28, a. m.	4¾	June 1, p. m.	4½	June 8, a. m.	6½	15¾
Do.	May 27, a. m.	3¾	May 31, a. m.	4	June 8, p. m.	8½	16¼
May 24, a. m.	May 28, a. m.	4	June 2, a. m.	5	June 9, a. m.	7	16
June 5, a. m.	June 11, a. m.	6	June 14, p. m.	3½	June 20, a. m.	5½	15
Do.	June 12, a. m.	7	June 16, a. m.	4	June 22, a. m.	6	17
Do.	do	7	June 15, a. m.	3	June 23, a. m.	8	18
Do.	June 10, p. m.	5½	June 13, p. m.	3	June 21, a. m.	7½	16
Do.	June 12, a. m.	7	June 14, p. m.	2½	June 20, a. m.	5½	15
Do.	June 10, p. m.	5½	June 13, a. m.	2½	do	7	15
Do.	June 12, a. m.	7	June 14, a. m.	2	June 21, a. m.	7	16
Do.	June 10, p. m.	5½	do	3½	June 19, p. m.	5½	14½
Do.	June 11, a. m.	6	June 14, p. m.	3½	June 20, a. m.	5½	15
Do.	June 10, a. m.	5	June 13, a. m.	3	June 19, a. m.	6	14
Do.	June 10, p. m.	5½	June 14, a. m.	3½	June 19, p. m.	5½	14½
Do.	June 11, a. m.	6	June 15, a. m.	4	do	4½	14½
Do.	June 11, p. m.	6½	June 14, a. m.	2½	do	5½	14½
Do.	June 11, a. m.	6	June 13, a. m.	2	June 18, a. m.	5	13
Do.	do	6	June 14, a. m.	3	June 19, a. m.	5	14
Do.	June 11, p. m.	6½	June 15, p. m.	4	June 24, a. m.	8½	19
Do.	do	6½	June 14, a. m.	2½	June 20, p. m.	6½	15½
Do.	June 13, a. m.	8	June 16, p. m.	3½	June 25, a. m.	8½	20
June 16, a. m.	June 20, p. m.	4½	June 24, p. m.	4	June 29, m.	4¾	13¼
Do.	June 21, a. m.	5	June 24, a. m.	3	June 29, a. m.	5	13
Do.	do	5	do	3	June 30, p. m.	6½	14½
June 17, a. m.	June 22, a. m.	5	June 25, p. m.	3½	July 2, a. m.	6½	15
Do.	June 23, a. m.	6	do	2½	do	6½	15
June 29, a. m.	July 3, p. m.	4½	July 8, a. m.	4½	July 16, p. m.	8½	17½
Do.	July 5, a. m.	6	July 9, a. m.	4	July 15, a. m.	6	16
June 30, a. m.	July 4, a. m.	4	July 8, a. m.	4	July 14, a. m.	6	14
Do.	July 3, a. m.	3	July 7, a. m.	4	July 12, a. m.	5	12
Do.	July 4, a. m.	4	do	3	July 13, a. m.	6	13
Do.	July 6, a. m.	6	July 9, p. m.	3½	July 15, p. m.	6	15½
Do.	July 4, a. m.	4	July 7, p. m.	3½	July 12, p. m.	5	12½

Total number of larvae, 50; average length of first instar, 5.48 days; average length of second instar, 3.67 days; average length of third instar, 6.70 days; average length of larval stage, 15.85 days.

DURATION OF THE LARVAL STAGE.—The length of the larval stage seems to depend mainly upon the temperature conditions. While in midsummer the stage may not require more than 11 days, in the early spring records of periods as long as 41 days have sometimes been obtained. Records obtained at Quincy, Fla., May 12 to July 16, 1922, are concisely set forth in Table II, which shows for each of 50 larvae the relative length of the three instars and the complete larval stage.

THE PUPA

When first transformed, the pupa is white in color and of the same approximate size as the beetle. During the resting stage the development can be plainly traced and the approximate age of the pupa readily determined. In summer the stages of development are as follows: First day, white all over; second day, compound eyes apparently dotted all over with reddish-brown; third day, all of compound eyes reddish-brown and tips of mandibles beginning to show a slight reddish color; fourth day, eyes almost black, tips of mandibles a dark reddish-brown, femur-tibial joint of each leg yellowish-brown, tarsi of legs very light brown, and entire body has taken on a dirty grayish appearance with the exception of the abdomen, which is still white; fifth day, parts showing color on the fourth day gradually darken until imago appears.

The length of the pupal stage is much shorter than that of the larval stage, but is also dependent to a certain extent upon temperature, and probably moisture, conditions. Pupal records obtained at Quincy, Fla., at various seasons of the year show a duration of from 4 to 11 days. The average length of this stage during the summer is approximately 5 days. The pupal record of 40 individuals taken at Quincy, Fla., May 28 to July 21, 1922, is shown in Table III.

THE ADULT

FEEDING HABITS.—When transformation to the adult stage has taken place, the beetles lie inactive within their cells from one to two days. Emergence then takes place, after which they rest for a period of about 24 hours. For several days after emergence the young beetles can be distinguished from those of the older generation by the fact that they are lighter and brighter in color and smaller in size. When the resting period has been completed, they commence feeding on the lower tobacco leaves which rest on the ground. In most of this early feeding the beetles confine their eating to the lower leaf surface. In a short time, however, the feeding punctures go entirely through the leaf.

TABLE III.—*Length of pupal stage of the tobacco flea-beetle at Quincy, Fla., May 28 to July 21, 1922*

Date of pupation	Date adult appeared	Length of pupal stage
		Days
May 28, p. m.	June 4, p. m.	7
May 29, p. m.	June 5, p. m.	7
May 30, a. m.	ditto	6½
June 3, a. m.	June 8, p. m.	5½
June 7, a. m.	June 12, a. m.	5
Do	ditto	5
Do	June 12, p. m.	5
June 8, a. m.	ditto	4½
Do	June 13, a. m.	5
June 8, m.	ditto	4¾
Do	ditto	4¾
June 8, p. m.	ditto	4½
June 9, a. m.	ditto	4
Do	ditto	4
Do	June 13, p. m.	4½
June 10, a. m.	June 14, a. m.	4
June 18, a. m.	June 23, a. m.	5
June 19, a. m.	June 24, a. m.	5
Do	June 24, p. m.	5½
June 19, p. m.	June 24, a. m.	4½
Do	June 24, m.	4¾
Do	ditto	4¾
Do	June 25, a. m.	5½
June 20, a. m.	ditto	5
Do	ditto	5
Do	ditto	5
June 20, p. m.	June 26, a. m.	5½
Do	ditto	5½
June 21, a. m.	June 25, p. m.	4½
Do	June 26, a. m.	5
June 29, a. m.	July 4, a. m.	5
June 30, p. m.	July 5, p. m.	5
July 2, a. m.	July 7, a. m.	5
July 12, a. m.	July 16, p. m.	4½
Do	July 17, a. m.	5
July 12, p. m.	July 18, a. m.	5½
July 13, a. m.	July 18, p. m.	5½
July 13, p. m.	ditto	5
Do	July 19, a. m.	5½
July 14, a. m.	July 21, a. m.	7
Average of 40 records of length of pupal stage		5.11

The beetles remain on the lower leaves for three or four days and then gradually spread over the entire plant. In the case of unshaded tobacco the feeding is largely confined to the lower portion of the plant. In the case of shaded tobacco, however, the upper leaves are frequently punctured to a considerable extent and even the bud leaves are sometimes fed upon. Under shade conditions there seems to be little choice between the upper and lower surfaces of the leaves, although in high tobacco there is apparently some slight preference for the upper surface. Numerous observations have shown that the beetles feed at night as well as during the day.

While tobacco is the preferred food plant of the tobacco flea-beetle, many other plants, wild and cultivated, belonging to the Solanaceae are fed upon to a considerable extent. In the absence of solanaceous plants, the beetles will feed sparingly upon various other wild and cultivated plants.

PREOVIPOSITION PERIOD.—On May 8, 1921, two adults emerged from pupal cells and were immediately transferred to an oviposition cage. On May 23 the first eggs were deposited, giving a preoviposition period of 15 days. Again, on June 25, 1922, six tobacco flea-beetles, which had just emerged from their pupal cells, were placed in an oviposition cage. No eggs were deposited in this cage until July 17, an interval of 22 days. Other experiments also bear out the fact that there is a period of about two to three weeks after emergence from the pupal cell before eggs are deposited.

OVIPOSITION PERIOD.—Deposition of eggs seems to begin very soon after emergence from hibernation. Indeed, if the weather is mild there are indications that a few scattering eggs may be deposited through the winter. At least one record has been obtained of eggs deposited on February 3.

From the middle of March to late summer deposition is fairly constant. The number of eggs laid may fluctuate more or less, judging from the appearance of the different generations, but since to a certain extent the generations invariably overlap, some eggs are always being deposited. After the tobacco is harvested, however, flea-beetles gradually become very scarce. There seems to be little activity in late summer or early fall, and after the beginning of September the deposition of eggs seems nearly to cease.

PROPORTION OF SEXES.—Of 407 overwintered beetles collected in early spring at Quincy, Fla., in 1922 and 1923, 68 per cent were females and 32 per cent males. In 1922, of 500 beetles of the spring generation collected in the field, 65 per cent were females and 35 per cent males. Of 500 beetles of the first summer generation collected during the same season, 52 per cent were females and 48 per cent males. These data show a predominance of females over males, especially in the overwintering and the spring generations.

MATING.—Mating was commonly observed at all hours of the day. Just how much time elapses between emergence of the adult from the pupal cell and the act of mating is not known, but it is probable that mating occurs during the first week.

Although several attempts were made, no eggs were obtained from unfertilized females.

DISPERSION.—The tobacco flea-beetle utilizes all three of the common methods of locomotion — crawling, jumping, and flying. Perhaps most of the movement from plant to plant in the field is done by the first two of these methods, but the flea-beetle is quite capable of sustained flight. Seed-beds have been known to become infested with this pest when the bed was located in the midst of heavy timber a mile from where any tobacco had been planted previously. When a seed-bed is located near a tobacco shade, beetles migrate readily from the bed to the shade. Numerous records have been made of the collection of this beetle on fly-paper screens 5 and 6 feet above the ground. It is scarcely conceivable that the tobacco flea-beetle should be capable of flying many miles, but that they can and do fly several hundred yards is unquestionable.

LONGEVITY.—Just how long a tobacco flea-beetle may live under normal conditions is largely a matter of conjecture. In the field the overwintered beetles have largely disappeared by the end of April. With the exception of the overwintering individuals, however, the average length of life under field conditions appears to range approximately from 40 to 50 days. Laboratory records obtained over a period of several years indicate that the average length of life in confinement somewhat closely follows that in the field.

Several records have been obtained of a few flea-beetles living for an abnormally long time. In one case where 200 overwintered beetles were placed in confinement April 1, the last beetle died August 14. Of another lot of adults placed in confinement about the middle of March, the last individual died July 5. In a third instance, 200 freshly-emerged adults of the spring generation were placed in confinement April 17. The last individual died September 25. This beetle had a definitely known longevity of 161 days. These records show that it is possible for a few individuals of one generation to live so long that their lives overlap two following generations.

NUMBER OF GENERATIONS.—There are usually four fairly well-defined generations in the region of Quincy, Fla.: The overwintering generation, which generally appears about the time tobacco plants are up in the seed-beds;

the spring generation, which emerges from about May 1 to May 15, when tobacco is from 12 to 18 inches high; the first summer generation, which appears from the middle to the end of June, when tobacco is nearly mature; and a second summer generation, which may appear upon late tobacco during the latter part of July or the early part of August.

In addition to the four broods mentioned, there are probably one or two more partial generations, but their numbers are so few and scattered, and the preceding generations so overlap, that it is impossible to make a definite statement concerning them.

HIBERNATION.—The tobacco flea-beetle passes the winter in the adult stage. In northern Florida this period is passed in an incomplete state of hibernation. During cold weather the beetles seek shelter under piles of leaves, weeds, trash, or any kind of material which affords protection. During warm periods, however, they may become active and are often to be found feeding in sheltered spots. During unusually warm weather breeding may take place to a limited extent.

SUMMARY

The tobacco crop in the southern cigar-wrapper district is attacked by the overwintered brood and by two later generations of the tobacco flea-beetle, *Epitrix parvula*. After the crop is harvested there may be two and possibly three additional broods, which so overlap that it is impossible to tell them apart.

The eggs of this beetle are usually deposited in cracks or crevices in the soil near the base of the plant.

Females of the overwintered brood may lay as many as 200 eggs, but the deposition in later broods is much less.

Moist soil, when available, is always selected for deposition in preference to dry soil. When moist soil is unavailable, the beetles deposit eggs very sparingly, even over long periods of time.

Egg deposition, as well as feeding, may take place at any time during the day or night.

In the early spring months at Quincy, Fla., incubation requires about 11 days, whereas in summer the eggs usually hatch in about 5 days. A certain amount of moisture is necessary for incubation.

The larvae of the tobacco flea-beetle live underground and feed upon the roots of the tobacco plant and other plants of the family Solanaceae.

Larval records obtained at Quincy, Fla., show that the length of this stage may vary from 11 days in midsummer to 41 days in early spring.

The pupal stage is passed in a small oval-shaped cell about an inch below the surface of the ground. The average length of this stage during the summer is about five days.

Newly-emerged flea-beetles remain near the ground and confine their feeding to the lower tobacco leaves. In a short time, however, they spread over the entire plant and feed on all portions of the foliage.

While tobacco is the preferred food plant of the tobacco flea-beetle, other plants, both wild and cultivated, belonging to the Solanaceae are fed upon to a considerable extent. In the absence of solanaceous plants the beetles will feed sparingly upon various other wild and cultivated plants.

Deposition of eggs commences soon after emergence of the beetle from hibernation and continues until early fall.

The tobacco flea-beetle is capable of flying a considerable distance, this being the most important means of dispersion.

Hibernation in the southern cigar-wrapper district is incomplete.

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THE DIFFERENTIATION OF PRIMARY ISOLATIONS OF BACTERIUM MELITENSIS FROM PRIMARY ISOLATIONS OF BACTERIUM ABORTUS (BOVINE) BY THEIR CULTURAL AND ATMOSPHERIC REQUIREMENTS [1]

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INTRODUCTION

That the resemblance of *Bacterium abortus* to *Bact. melitensis* in morphological, cultural, biochemical, and serological respects is so close that their differentiation is difficult if not wellnigh impossible was discovered by Alice C. Evans (*6*)[2] in 1918, and later confirmed by Feusier and Meyer (*8*). This has led investigators to wonder whether the likeness in the two organisms ceases with form, action, and appearance upon artificial culture media and serological characteristics, or involves pathogenicity as well. Investigations bearing upon pathogenicity made later by Fleischner, Vecki, Shaw, and Meyer (*9*) indicate that the similarity even with respect to pathogenicity may be less remote than formerly suspected. Further information on this phase of the subject has been contributed by Khaled (*18*), Huddleson (*16*), Evans (*7*), and others.

In view of the fact, however, that severe illness in humans has never been definitely traced to the ingestion of *Bacterium abortus* in milk, few investigators have considered the two organisms identical; and the practice of utilizing milk containing this microorganism is far too general to permit ignoring the great quantity of available evidence thereon. Comparative studies of *Bact. abortus* and *Bact. melitensis* seem to have been mainly confined to strains which have been under artificial cultivation for considerable periods of time and are well accustomed to propagation upon artificial media rather than to those of recent isolation.

During 1922 and 1923 the writer had opportunity to make comparisons of some recently isolated strains of the two types of organisms. Results of this work seem to indicate that by cultural methods *Bacterium melitensis* and *Bact. abortus* (bovine) may be readily distinguished during their primary isolations.

The cultural results differed but little from those anticipated. Investigators of Malta fever have written little if at all of special or unusual biological requirements of the Malta fever organism, whereas in bovine infectious abortion the peculiar cultural characteristics of *Bacterium abortus* seem to have been the factor which retarded its discovery. The originality manifested by Bang and Stribolt (*1*) in their successful cultural work with *Bact. abortus* has never ceased to receive the profound respect which it rightfully deserves.

EXPERIMENTS AND RESULTS

During August, 1922, 24 samples of blood serum from goats suspected of being affected with Malta fever were forwarded to the Pathological Division, Bureau of Animal Industry, by Dr. W. A. Heck, a practicing veterinarian of Donna, Hidalgo County, Tex. When the samples were subjected to the agglutination test for this disease, four reacted in a dilution of 1 to 1,000, one 1 to 500, seven 1 to 200, two 1 to 100, one 1 to 50, and one 1 to 25. Eight gave negative results to the test in a dilution of 1 to 25 or higher. About a month before these samples arrived, T. B. Hooks, of Donna, Tex. (owner of the animals), had inquired if any test could determine whether the goats were affected with Malta fever. He stated in the communication that his brother, who had been using milk from this flock, had been suffering six months with some kind of fever suspected of being Malta

[1] Received for publication June 3, 1924; issued March, 1925.
[2] Reference is made by number (italic) to "Literature cited," pp. 590–591.

fever. The animals were described as fine Nubian milk goats costing from $200 to $600 each and the flock was mentioned as consisting of about 100 head. Sterile containers were shortly afterward forwarded to Doctor Heck with the request that he obtain from some of the higher reacting goats specimens of blood, milk, and urine for cultural and inoculation work. The reactors had been destroyed before the request was received; consequently no further action was taken at that time.

About two months later, W. E. Whigham, of Donna, Tex., wrote asking permission to forward for the application of the agglutination test for Malta fever blood-serum samples from two flocks of goats, approximately 100 head each, which had been exposed to the flock previously tested. Doctor Whigham stated that he had had three or four cases of Malta fever in human beings contracted from drinking the milk of these goats, one of his patients being the owner of one of the exposed flocks.

ORIGIN AND METHOD OF ISOLATING BACTERIUM MELITENSIS

On November 23 and 25, 212 more serum samples from the two flocks mentioned were received. Of this number three reacted to the agglutination test for Malta fever in a dilution of 1 to 1,000, one 1 to 500, nine 1 to 200, eleven 1 to 100, seven 1 to 50, and eight 1 to 25. The remaining samples were negative in dilutions of 1 to 25 or higher.

On November 6 a sample of human blood was received from Doctor Whigham. It was described as being obtained from one of his patients, a Mexican goat herder, 14 years of age, who had reacted to the Malta-fever test. The boy had been suffering with fever about 9 months, more or less continuously, his temperature reaching 102 to 103° F. in the afternoon. The specimen was somewhat decomposed upon arrival, but was sown on serum agar, subjected to the agglutination test both with a *Bacterium melitensis* and *Bact. abortus* antigen, and used for animal-inoculation purposes. The sample reacted in a dilution of 1 to 1,000 with both antigens.

In making the inoculations 11 guinea pigs were used; 6 were injected intra-abdominally with a physiological salt solution suspension of the blood and 5 both intra-abdominally and intratesticularly. Intratesticular inoculations had been described by Meyer and his coworkers (*21*) as an effective way of transmitting the disease through injections of *Bacterium melitensis* cultures. On December 22, between five and six weeks from the date of inoculation, five guinea pigs were destroyed. Two had received the material into both the abdomen and one testicle, and three had received it free in the abdominal cavity. Blood serum from these animals gave negative results to the agglutination test for Malta fever. Serum-agar slants sown with the spleen tissue remained sterile.

On December 28 the six remaining guinea pigs were destroyed. The autopsy findings and the cultural and serological results were as follows:

Guinea pig No. 77012. Inoculated intra-abdominally and intratesticularly. Spleen was somewhat thickened and surface irregular. Mesenteric lymph glands were congested. The inoculated testicles showed a constriction about midway between its extremities. On one side of the constriction was a small abscess containing semifluid pus. Other organs appeared normal. Cultures were made from the heart, liver, spleen, testicles, kidneys, and mesenteric lymph glands. Serum-agar slants alone were used. Blood serum reacted to the agglutination test with both *Bacterium abortus* and *Bact. melitensis* antigens in a dilution of 1 to 1,000. Its end point was not determined.

A portion of the cultures from each organ was incubated in closed jars in which 10 per cent of the air had been displaced by CO_2 gas and in which a ball of moistened cotton had been placed, providing conditions highly favorable for the growth of *Bacterium abortus*. Another lot was incubated under normal atmospheric conditions. The tubes in all cases were merely closed with cotton plugs, and the incubator was maintained at a temperature of 37° C. On December 30 the cultures were examined with no evidence of growth. On January 2, two cultures from spleen incubated under CO_2 conditions showed from 100 to 200 small opalescent colonies, two from affected testicle 15 to 20 colonies so small as to be recognized with difficulty, two from mesenteric lymph glands 10 to 15 colonies, one from kidney sterile, one from liver 3 colonies, one from heart sterile. Two cultures from spleen incubated under normal atmospheric conditions showed 200 to 300 colonies somewhat larger than those developing in a 10 per cent CO_2 atmosphere, one tube from testicle 50 to 75 colonies, one from liver 40 to 50

colonies, one from heart sterile. The organisms seem to have multiplied somewhat more rapidly in all cases under normal atmospheric conditions than in air partially displaced by CO_2 gas.

Guinea pig No. 77013. Inoculated both intra-abdominally and intra-testicularly The inoculated testicle showed a slightly abnormal appearance, probably at site of injection. An area about one-fourth inch in diameter was darker in color than the surrounding tissue. No other lesions were noted. The blood serum of this pig showed a titer of 1 to 50 with a *Bacterium melitensis* antigen and below 1 to 25 with a *Bact. abortus* antigen. Spleen and affected testicle cultured. Cultures were subjected to the same conditions as those of guinea pig No. 77012. On January 2, two cultures incubated under CO_2 conditions from spleen showed from 100 to 200 colonies; two tubes from testicle were sterile. The colonies developing under normal atmospheric conditions were slightly larger than those incubated in a 10 per cent CO_2 atmosphere.

Guinea pig No. 77014, inoculated into both testicle and abdominal cavity, and guinea pigs Nos. 77016 and 77107, which received the material intra-abdominally only, showed no lesions. Their blood serum did not agglutinate suspensions of either *Bact. abortus* or *Bact. melitensis* in dilutions of 1 to 25 or higher. Cultural results were also negative.

The colonies obtained in cultures from the two positive guinea pigs Nos. 77012 and 77013 closely resembled those of *Bacterium abortus*, but failed to attain, in four days, the size commonly reached by such colonies on serum-agar slants when incubated in moist jars containing 10 per cent CO_2 gas. A few of the larger colonies were approximately one-sixteenth inch in diameter. In most cases they were considerably smaller. On January 16 subcultures were made from the original spleen and testicle cultures on 3 per cent glycerin-agar slants. Slight growth was visible on the day after incubation under normal atmospheric conditions. On the second day the growth was reasonably heavy. Antigens prepared from subcultures of the organisms isolated from guinea pigs Nos. 77012 and 77013 were agglutinated in a characteristic manner by *Bact. abortus* and *Bact. melitensis* antiserums.

Efforts failed to isolate *Bacterium melitensis* from the blood of the 14-year-old boy by cultural methods. The serum-agar slants sown with different dilutions of the blood were heavily overgrown with contaminating organisms within a few hours.

On December 14, through the kindness of Doctor Heck, blood specimens were obtained from four of the goats, Nos. 96, 26, 61, and 19, exposed to the original reacting flock. Milk samples were also secured from three of these. The agglutination titer of the blood serums of goats No. 96, 26, and 61 with a *Bacterium melitensis* antigen was 1 to 1,000; goat 19, 1 to 500. The milk whey of goat No. 96 was negative; goat No. 26, titer 1 to 1,000; goat No. 19, titer 1 to 100.

Different dilutions of the blood and milk were sown on serum-agar slants and incubated under CO_2 and normal atmospheric conditions. On some of the tubes there developed colonies bearing rather close resemblance to those of *Bacterium abortus* and *Bact. melitensis;* but subcultures of these failed to produce antigens that were agglutinated by *Bact. abortus* and *Bact. melitensis* antiserums.

Four guinea pigs received 2 c. c. of milk each from three of the goats and the same number received suspensions of the blood of each blood sample. The milk was somewhat putrid on arrival. All the guinea pigs receiving the milk of goats Nos. 19 and 96 were dead in less than 48 hours.

Guinea pig No. 76595, inoculated with the blood of goat No. 61, died December 22. Cultures from spleen, liver, and kidneys remained sterile.

Guinea pig No. 77411, inoculated with the blood of goat No. 26, died December 30. Cultures from the liver and spleen remained sterile.

Guinea pig No. 77496, inoculated with milk from goat No. 26, died December 31. Cultures from liver and spleen developed colonies of *Bacillus coli;* no *Bacterium abortus*-like colonies were detected.

Guinea pig No. 77497, inoculated with the milk of goat No. 26, was destroyed January 29. Spleen was three times normal size. Liver showed evidence of fatty degeneration. Titer of blood serum to *Bacterium melitensis* antigen was 1 to 200; to *Bact. abortus* antigen, 1 to 200.

Guinea pig No. 77498, inoculated with the milk of goat No. 26, was destroyed January 29. Spleen was slightly enlarged. Right epididymis showed evidence of necrosis. Titer of blood serum to both *Bacterium melitensis* and *Bact. abortus* antigens was 1 to 500. Serum-agar slants, 3 per cent glycerin-agar slants, and infusion bouillon were used for culturing the spleens and testicles of guinea pigs Nos. 77497 and 77498.

COMPARISON OF CULTURAL AND ATMOSPHERIC REQUIREMENTS OF BACTERIUM ABORTUS (BOVINE) WITH THOSE OF BACTERIUM MELITENSIS

When the isolation of *Bacterium melitensis* through guinea-pig inoculations were being attempted, the writer was also testing the efficacy of abortion-bacterin treatment as a means of eliminating *Bact. abortus* from udder-infected cows. Sixteen cows, all cases of natural infection, were being used in this experiment. Three or four guinea pigs were injected with 5 c. c. quantities of milk from each cow at brief intervals and later destroyed, subjected to the agglutination test for abortion disease, and cultured with the object of determining whether any of the cows had ceased to be carriers of the microorganism or were still producing milk infected by *Bact. abortus.*

On January 29, when guinea pigs Nos. 77497 and 77498 were destroyed and cultured, 43 guinea pigs previously inoculated with milk of the above-mentioned cows were also destroyed, autopsied, cultured, and subjected to the agglutination test for abortion disease. Cultures were made from the spleens of all the animals, using for medium the lot of serum-agar slants employed for culturing guinea pigs No. 77497 and 77498. Enough slants were inoculated with the spleen of each guinea pig to allow the incubation of a portion of the cultures under both CO_2 and normal atmospheric conditions. The suspected *Bacterium melitensis* cultures were placed in the same jars containing 10 per cent CO_2 as the *Bact. abortus* cultures. In other words, cultural conditions were identical.

Examination of the cultures incubated under normal atmospheric conditions on January 30 and January 31 failed to reveal any evidence of growth. On February 1 the tubes subjected to both methods of incubation were examined. Serum-agar slants from spleen of guinea pig No. 77497 showed small opalescent colonies under both methods of cultivation. Likewise serum-agar slants from testicle and spleen of guinea pig No. 77498 showed small colonies under both conditions. There appeared to be little or no difference in size of colonies under both methods of incubation.

Thirty-five serum-agar slants from spleens of the abortion experiment guinea pigs incubated in closed jars with 10 per cent CO_2 showed opalescent colonies indicative of *Bacterium abortus.* The colonies were considerably larger than those suspected of being *Bact. melitensis.* Under normal atmospheric conditions a like number of tubes showed no evidence of growth, although sown with spleen tissue of these same guinea pigs. The cultures were further subjected to the same conditions of incubation, and on February 5 the colonies developing from inoculations of guinea pigs Nos. 77497 and 77498 on serum-agar slants had increased considerably in size. The tubes under the two methods of incubation differed but slightly, if at all. Colonies from the inoculations of these guinea pigs were visible on 3 per cent glycerin-agar slants, and the infusion-bouillon cultures plainly showed evidence of rather luxuriant growth. The colonies in the closed jars developing from the abortion experiment guinea pigs appeared considerably larger than those from the *Bact. melitensis* guinea pigs. Under normal atmospheric conditions the serum-agar slants from the abortion experiment guinea pigs appeared sterile and remained so until February 12, when they were discarded.

While guinea pig No. 77497 showed well-marked lesions, the number of colonies developing from the spleen inoculations was less than 25 on any of the tubes of solid medium. Inoculations from the spleen of guinea pig No. 77498 resulted in the isolation of from 100 to 200 colonies each, although the lesions here were not pronounced.

Antigens prepared from the infections isolated from guinea pigs Nos. 77497 and 77498 were agglutinated by *Bacterium abortus* and *Bact. melitensis* antiserums in such a manner as to indicate that either *Bact. abortus* or *Bact. melitensis* was being dealt with.

Bacterium abortus was isolated from 20 of the 43 guinea pigs employed in the abortion experiment when the cultures were incubated in a partial CO_2 atmosphere. It is probable that numerous strains were represented, since in this test eight different cows used in the experiment were found to be still carrying the infection in their milk. Since numerous workers have observed that strains of *Bact. abortus* which have become accustomed to artificial media even after being injected into the bodies of bovines and multiplying for long periods can be readily cultivated again under normal atmospheric conditions, it may be well to state that no abortion vaccine had ever been used, nor artificial infection of any animals with *Bact. abortus* practiced in the herd where the abortion experiment was being conducted.

On February 14, when the remaining 15 guinea pigs, 14 of which had been inoculated with blood from the four goats, were destroyed, no lesions indicative of *Bacterium melitensis* infection were detected. Negative agglutination and negative cultural results for *Bact. melitensis* were obtained.

Since the isolation of the Texas strains of *Bacterium melitensis*, the writer has endeavored to determine with what frequency *Bact. abortus* may be isolated from the spleens of infected guinea pigs, following their inoculations with *Bact. abortus* infected milk, when sown on serum-agar slants in cotton-plugged tubes and placed in an incubator kept moist by the introduction of a receptacle containing water. Success has been attained only when the guinea pigs were inoculated with milk from cows which had been artificially infected with *Bact. abortus* strains accustomed to propagation in a normal atmosphere, although such tests have numbered between 75 and 100. Sealing the tubes after heating their upper portions caused isolation of the organism in some cases, but growth was slow as compared with the carbon-dioxid method.

THE WORK OF SOME PREVIOUS INVESTIGATORS

Bruce (*2*), the discoverer of *Bacterium melitensis*, in announcing his discovery makes no mention of using special medium or of incubating his cultures in other than the usual manner. Agar-agar nutrient jelly is recorded as having been used for medium. Some of his tubes sown with spleen tissue of a fatal human case were left at room temperature, and some subjected to 37° C. incubation. Under 37° incubation, growth was visible in 68 hours. At room temperature 168 hours elapsed before growth was observed.

Horrocks (*13*) describes the isolation of *Bacterium melitensis* from the milk of a goat by spreading milk sediment on litmus-nutrose-agar plates. After four days incubation at 37° C. colonies of the organism appeared on all the plates sown.

Shaw (*23*) describes investigational studies of 91 goats. The milk sediment of 30, which gave positive agglutination reactions, was sown on nutrose-litmus-agar plates. *Bacterium melitensis* was isolated from 7 of the number following incubation at 37° C. He further describes serological and cultural work in connection with 33 cows on the island of Malta. Ten of the number gave agglutination reactions varying from 1 to 30 to 1 to 1,000. From the milk sediment of 2 of this number *Bact. melitensis* is mentioned as having been repeatedly isolated, 3 plates yielding in some cases more than 200 *Bact. melitensis* colonies. The method described in the isolation of the organism consisted in centrifugalization of the milk samples and the sowing of the sediment on nutrose-litmus-agar in Petri dishes. Examination of the cultures was made after five days' incubation at 37°.

Keefer (*17*), in referring to the results of Shaw, suggested that Shaw was probably dealing with *Bacterium abortus*. The writer's experience has been that in the isolation of the abortion organism from the milk of naturally infected cows considerable difficulty is encountered in obtaining the original cultures, even when seemingly ideal conditions were provided. These observations and the results reported by different workers in this connection lead to the assumption that Shaw's conclusions may have been wholly correct, regardless of the fact that at the time the Malta fever investigations were made the close similarity in morphology, biochemical, and serological characteristics of the Malta fever and abortion organism was little suspected.

Huddleson (*15*), who deserves credit for greatly simplifying the method of isolation of *Bacterium abortus* from infected tissues and fluids, and the discovery that CO_2 gas in combination with air in the proper proportion is the essential factor rather than the degree of oxygen tension, states that to simplify the isolation of the organism from milk the following factors must be considered:

> The medium and its proper preparation; the proper H-ion concentration of the medium; the employment of an agent which will eliminate fast-growing organisms and the method of incubation.

Cooledge (*3*) in discussing the isolation of *Bacterium abortus* from milk of infected bovines calls attention to the defects of the Nowak method, in which cultures of *Bacillus subtilis* are grown in closed jars with those sown with suspected *Bact. abortus* infected materials. While he mentions having succeeded in isolating *Bact. abortus* through the use of this method he refers to it as—

> too tedious a process for application to a considerable number of samples, requiring weeks to isolate and identify the cultures,

a feature which prompted him to investigate the value of the agglutination and complement-fixation tests in connection with milk whey as a means of detecting *Bact. abortus* udder-infected cows.

Giltner, Huddleson, and Tweed (*10*), writing on *Bacterium abortus* udder infection in bovines, refer to the cultural requirements of the organism in milk, and ascribe Huddleson's high degree of success in its primary cultivation from the milk to the probability that a 10 per cent CO_2 atmosphere, which he utilized, exists within the udder, since, according to their statement—

> freshly drawn milk from the healthy udder shows approximately this pressure of CO_2.

Meyer and Shaw (*20*), on the contrary, seem of the opinion that the peculiar requirements of the abortion organism are observed only from uterine isolations, and refer to the results of McFadyean and Stockman (*19*), Holth (*12*), Schroeder and Cotton (*22*), Evans (*5*), and Steck (*24*) to substantiate their view.

Huddleson (*14*) has shown that sealed tubes slowly develop a 10 per cent CO_2 tension, the colonies developing when this is reached. Cooledge (*3*) refers to the claims of certain investigators that when tubes of agar are sealed and incubated there is a period when the proper amount of oxygen is present, owing to a partial absorption of oxygen by the agar. Some of the confusion regarding this feature may perhaps be ascribed to the fact that in the earlier investigations the writers may have considered certain details in the technic too inconsequential to mention, or that in the studies the investigators failed to take into consideration whether they were dealing with laboratory strains or with those which had never become accustomed to growth on artificial media. The writer was recently informed by Dr. W. E. Cotton that in the early isolations of the organisms from pigs infected with market milk, recorded by Schroeder and Cotton (*22*), sealing of the tubes was practiced, and that development of colonies required an incubation period of from a week to ten days.

Original cultures of *Bacterium abortus* obtained from swine appear to show different atmospheric requirements. Differentiation of porcine strains of *Bact. abortus* from *Bact. melitensis* by their cultural or atmospheric requirements may be difficult or even impossible. Good and Smith (*11*), in describing their success in isolating *Bact. abortus* from a case of swine abortion by the Nowak method, mention having observed the development of colonies on the fourth day. "The first subcultures," they state, "grew readily in the air, whereas subcultures derived from the cow usually do not grow in the air until after being cultured for several generations by the Nowak method." Since none of the original cultures were incubated in the air, they were unable to state whether growth would have taken place.

Doyle and Spray (*4*) find results seeming to indicate that the atmospheric requirements of the porcine organism may differ from those of *Bacterium abortus* (bovine), since they mention having isolated the organism after three days' incubation on serum-agar slants from guinea pigs inoculated with an emulsion of the placenta of a naturally infected sow. No mention is made of having subjected the cultures to other than normal atmospheric conditions.

SUMMARY

The isolation of *Bacterium melitensis* from guinea pigs infected by inoculations with blood from human cases of Malta fever, or with milk from goats affected with the disease, may be readily accomplished when serum-agar slants are sown with the infected tissues and incubated under normal atmospheric conditions at 37° C.

The original cultures develop with equal if not greater rapidity in a normal atmosphere than in one partially displaced by CO_2 gas. This characteristic permits the differentiation of *Bacterium melitensis* and *Bact. abortus* (bovine) in primary isolations.

Since artificially cultivated strains of *Bacterium abortus* do not promptly become exacting in their atmospheric requirements when again introduced into animals, the biological characteristics of such strains may be identical with those of *Bact. melitensis*.

The results of different investigators would suggest that the differentiation of *Bacterium melitensis* from porcine strains of *Bacterium abortus* may also be impossible by observations of their biological characteristics.

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FEED COST OF MILK PRODUCTION AS AFFECTED BY THE PERCENTAGE FAT CONTENT OF THE MILK [1]

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This paper is an elaboration of a subject which was touched incidentally by Gaines and Davidson (*3, p. 596*)[2] in a study of the relation between percentage fat content and yield of milk. In the last analysis feed cost in this paper refers to financial cost. Approach is made, however, through certain physiological principles and relationships which permit deductions as to feed cost in terms of nutrients.[3] From feed cost in terms of nutrients to relative feed cost in terms of dollars is a comparatively simple and direct step in the present case.

From a nutritional standpoint the feed cost of producing milk falls in two categories: (1) Maintenance requirements, that is, the nutrients required by the cow as not lactating; (2) lactation requirements, that is, the *additional* nutrients required as lactating. The term "maintenance" as here used includes the requirements for all metabolic activities except those associated with lactation. Such usage is common in our present-day feeding standards for cows in milk.

According to our feeding standards the nutrients required for maintenance and for lactation are approximately equal in the case of an 1,100-pound cow producing 9,277 pounds of 4 per cent milk per year. For a lower yield the maintenance requirement is the larger and for a higher yield the lactation requirement is the larger. Roughly speaking, in very good dairy practice the two categories of feed cost—maintenance and lactation—are of equal weight. Together they constitute the feed cost of milk production, and the problem is to express them as a function of the percentage fat content of the milk. We may consider, first, the lactation requirements; second, the maintenance requirements; third, the milk-production requirements; that is, the sum of the lactation and maintenance requirements; and fourth, the translation of this sum into terms of dollars.

THE LACTATION REQUIREMENTS

In the development of feeding standards the lactation and maintenance requirements were early distinguished. Haecker carried the development further by showing experimentally that the lactation requirements are dependent on the composition of the milk as well as on the amount of milk. Haecker (*4, p. 50*) made the fundamental observation " * * * that there was a remarkable uniformity in the net nutriment required for the production of a unit of milk solids when nutrients consumed and milk solids produced were reduced to carbohydrate equivalent." He accordingly formulated his feeding standard on the basis of the composition of milks of varying fat percentage as indicated by analyses made in his own laboratory. His formulation on this basis has stood the test of several years of practical use and the scrutiny of considerable experimental investigation.

Haecker's observation quoted above is a key for attack of the lactation requirements. His "carbohydrate equivalent" of the milk solids is equivalent to energy value of the milk solids. And the energy value of milk solids in a given amount of milk may be simply expressed as a function of the fat percentage of the milk. If we take one pound as our unit amount of milk, and measure energy value in large calories, letting E represent energy value, and, t, percentage fat content of the milk we have,[4] $E=49.64\ (2.66+t)$. If Haecker's generalization is correct the nutrients required for lactation are directly proportional to $49.64\ (2.66+t)$, and may be expressed as $K_L\ (2.66+t)$ where K_L is a constant. His published data permit of detailed examination as to how well this generalization is supported by his experimental observations.

Haecker's published data pertain to 142 individual feeding trials with

[1] Received for publication April 1, 1924—issued March, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 601.
[3] The term "nutrients" as used throughout this paper refers to total digestible nutrients of the feed, digestible fat being multiplied by the factor 2.25.
[4] For the derivation of this equation see Gaines and Davidson (*3, p. 562*).

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an average length of 148 days, and include for each trial, and for each cow, expressed as a daily average: (1) Milk yield; (2) per cent fat (3) nutriment daily; (4) nutriment for maintenance; and (5) nutriment for product. The "nutriment daily" (corresponding to milk production requirements as used here) was estimated from the weights and analyses of the feeds consumed by the cow. The "nutriment for maintenance" was estimated from the live weight of the cow, allowing 7.92 pounds of nutrients per day per 1,000 pounds live weight (*4, p. 10*). The "nutriment for product" (corresponding to lactation requirements as used here) was estimated as "nutriment daily" minus "nutriment for maintenance." Haecker's data may be reworked and rearranged to better serve the present purpose. From his data the writer has calculated the lactation requirements *per pound of milk* and quite diverse also in breeding—Ayrshire, Brown Swiss, Guernsey, Holstein, Jersey, Shorthorn, crossbred, and native.

The mean nutrients requirement for lactation per pound of milk for each of the fat percentage classes has been derived from Table I and the data are shown graphically in Figure 1. It is evident at once from the figure that the nutrients for lactation may be expressed as a linear function of the fat percentage. We have now to determine a value for K_L, in a curve of the of the equation $N_L = K_L\ (2.66+t)$, for these particular data and see how its slope conforms to the observed results. The curve is simply fitted so that the algebraic sum of the deviations is zero, and this gives a value of 0.049 for K_L. The calculated values are given in Table II and graphically in Figure 1. The agreement with the observed values is very close indeed.

TABLE I.—*Correlation of the variables, percentage fat content of milk and nutrients for lactation per pound of milk* [a]

Pounds of nutrients per pound of milk-class mid-points	Percentage fat content of milk—class mid-points								
	2.55	3.05	3.55	4.05	4.55	5.05	5.55	6.05	Total
0.215	1	3	1	1					6
.245	1		2	1					4
.275	2	3	7	1	6	1	1		21
.305		6	3	3	6	3			21
.335		1	6	2	9	2			20
.365		1	2	3	7	6	4		23
.395	[b] 1			1	1	11	7		20
.425	[b] 1		2	2	1	3	5	2	15
.455					1	1	4	1	7
.485							2		2
.515						1			1
Total	4	14	23	14	31	28	23	3	140

$r = 0.6529 \pm 0.0327$

[a] Data from Minn. Bul. No. 140.

[b] These two records are from the same cow (Lou II), stated in the text (*p. 20*) to have been out of condition at the time. It has seemed advisable to exclude these records in the computations.

correlated this result with the per cent of fat in the milk. Table I shows the correlation surface.

The coefficient of correlation, 0.6529 ± 0.0327, is perhaps not so high as one might expect to find in a relation of this kind. It must be borne in mind, however, that there are several other variables involved here, such as variability in the estimated consumption of nutrients as compared with the actual (including loss of body tissue); and the estimated maintenance requirements as compared with the actual (including gain of body tissue). The 46 cows used in Haecker's experiments ranged in age from 2 to 14 years, and in weight from 593 to 1,347 pounds. They were

TABLE II.—*Lactation requirements per pound of milk as affected by percentage fat content of milk* [a]

Per cent of fat in milk (t)	Number of observations	Nutrients per pound of milk	
		Observed values	Calculated values, N_L [0.049 (2.66 $+t$)]
2.55	4	0.253	0.255
3.05	14	.286	.280
3.55	23	.310	.304
4.05	14	.333	.329
4.55	31	.333	.353
5.05	28	.380	.378
5.55	23	.409	.402
6.05	3	.435	.427

[a] Data from Minn. Bulletin 140.

Haecker's (*4*) standard and Morrison's (*5*) standard (mean of his range values) are included in the graph. It will be observed that Haecker's standard does not correspond as closely to his own observations as does Morrison's standard. Morrison's standard presumably represents his summary of all the available evidence on the relation.

The relation between the composition of the milk and the nutrients required for lactation, under comparable conditions of feeding, may be stated thus: *The nutrients required for lactation are directly proportional to the energy value of the milk solids.* This relation should be known as HAECKER'S LAW.

maintenance estimate strictly upon the weight of the cow. This is common scientific practice of the present time and we may assume that it is justified in the case of the milk cow.

One of the first questions to answer is, is there any correlation between size (maintenance requirements) of the cow and percentage fat content of the milk? Eckles (*2, p. 403*) is authority for the statement that "there is no relation within the breed between the size of the animal and the richness of the milk." Statistical evidence on this point is summarized in Table III. This table gives the mathematical constants of the correlations between certain size measurements (largely weight) of the

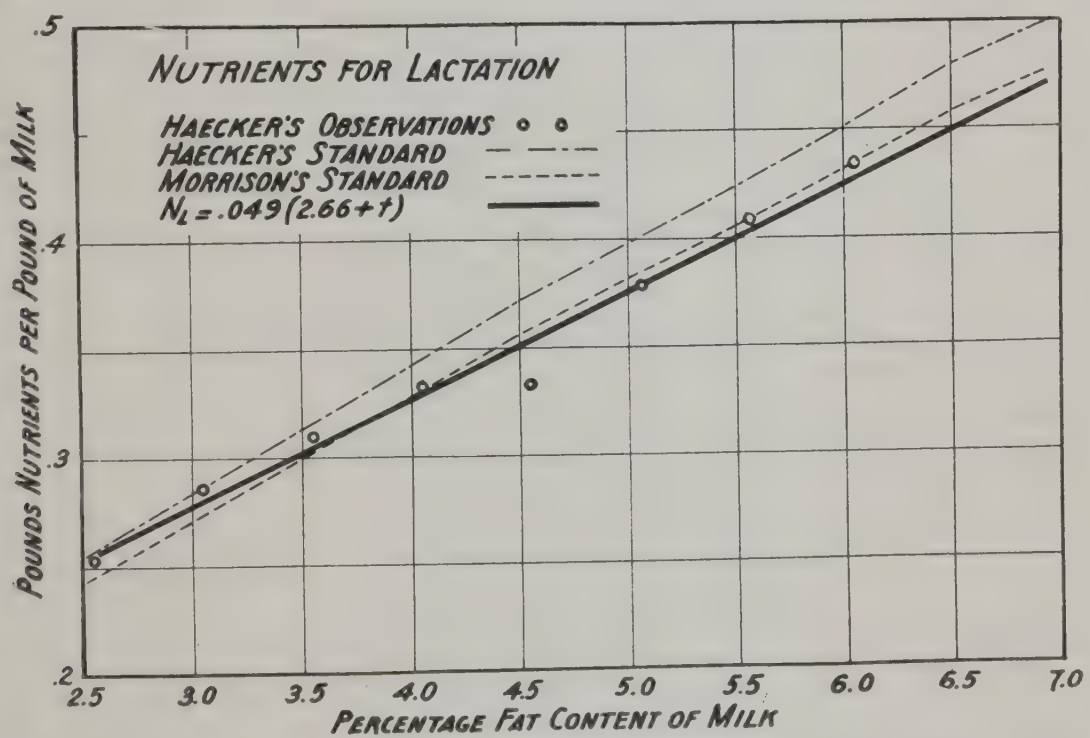

FIG. 1.—Lactation requirements per pound of milk as affected by percentage fat content of milk.

In the equation, $N_L=0.049\ (2.66+t)$, to the solid line curve, N_L=pounds of nutrients per pound of milk, and t=per cent of fat in the milk.

Coming back to the present viewpoint, it may be concluded, at least so far as commercial milk production is concerned, that the lactation requirements, N_L, may be expressed as a function of the fat percentage of the milk, t, by the equation:

$$N_L=K_L\ (2.66+t)$$

in which K_L is a constant *so far as affected by fat percentage.*

THE MAINTENANCE REQUIREMENTS

We have next to consider the maintenance requirements per unit of milk in relation to the percentage fat content of the milk. Haecker based his

cow and the percentage fat content of the milk as derived from published records indicated in connection with the table.

From Table III it is plain that weight and fat percentage are entirely independent within any one of the breeds represented. Therefore (within the same breed) the maintenance requirements are independent of fat percentage, and, *so far as affected by fat percentage*, the maintenance requirements are a constant, K_m. The maintenance requirements per pound of milk are, therefore,

$$\frac{K_m}{\text{milk yield in pounds}}$$

TABLE III.—*Mathematical constants of the correlations between size of cow and percentage fat content of milk* [a]

Breed and source of records [b]	Age of cow	Number of records	Size of cow, pounds or inches				Fat percentage			Coefficient of correlation
			Measurement	Mean	Standard deviation	Coefficient of variation	Mean	Standard deviation	Coefficient of variation	
	Years									
Jersey R. M	1	623	Weight	741.2± 2.5	90.7 ±1.7	12.2±0.24	5.448±0.015	0.568±0.011	10.4±0.20	0.008±0.027
Do	2	2,308	do	795.4± 1.3	92.6 ± .9	11.6± .12	5.457± .008	.546± .005	10.0± .10	− .037± .014
Do	3	1,348	do	857.6± 1.7	91.6 ±1.2	10.7± .14	5.438± .010	.531± .007	9.8± .13	− .001± .018
Do	4	1,068	do	899.5± 2.1	99.9 ±1.5	9.4± .14	5.427± .011	.527± .008	9.7± .14	.040± .021
Do	5	905	do	921.3± 2.2	95.9 ±1.5	10.4± .17	5.368± .011	.500± .008	9.3± .15	− .008± .022
Do	6	641	do	930.6± 2.6	99.2 ±1.9	10.7± .20	5.351± .015	.554± .010	10.4± .20	− .009± .027
Do	7	486	do	931.4± 3.0	98.5 ±2.1	10.6± .23	5.360± .016	.553± .012	9.9± .21	.019± .031
Do	8+	725	do	939.2± 2.4	94.5 ±1.7	10.1± .18	5.282± .013	.517± .009	9.8± .17	− .012± .025
Do	All ages	8,104	do	861.1± .9	114.6 ± .6	13.3± .07	5.409± .004	.538± .003	9.9± .05	− .041± .007
Guernsey W. C. C	do	124	do	979 ± 7.4	122.5 ±5.2	12.5± .54	5.080± .028	.467± .020	9.2± .39	.010± .060
Holstein W. C. C	do	145	do	1,234.6±10.3	184.4 ±7.3	14.9± .60	3.444± .016	.284± .011	8.2± .32	.001± .056
Holstein A. R. O	do	416	do	1,098.5± 5.5	167.6 ±3.9	15.2± .36	3.510± .012	.376± .009	10.7± .25	.088± .033
Do	do	416	Height	51.9± .07	2.20± .05	4.2± .10	3.510± .012	.376± .009	10.7± .25	.028± .033
Do	do	416	Length	61.8± .13	4.03± .09	6.5± .15	3.510± .012	.376± .009	10.7± .25	.067± .033

[a] The class intervals used in making up the correlation surfaces from which the data of this table are derived were as follows: For fat percentage, 0.1; for size, 50 pounds in weight, 1 inch in height at withers, and 2 inches in body length. The weights are for the most part estimates and there is a tendency to estimate at even 100 pounds; a lesser tendency, at 50 pounds, and occasionally, at 25 or 75 pounds. As an offset to this tendency the class ranges were chosen as 376–424, 425–475, 476–524, 525–575, etc. A similar plan was followed in the linear measurements to offset a tendency to even inches, to a lesser extent one-half inches, and occasionally one-quarter or three-quarters inches.

[b] The Jersey records are from the Register of Merit, vols. 1916, 1917, 1918, and 1919, all long-time records except R. M. No. 6394 (estimated weight 1950 pounds); the Guernsey and Holstein Wisconsin Cow Competition records are from the Wisconsin Cow Competition, Wisconsin Res. Bul. No 26; (long-time record); the Holstein Advanced Register O records are from the Advanced Register, vols. 12–17 inc., all 7-day records giving weight, shoulder height, and body length of cow.

The next question is as to the relation between fat percentage and milk yield. Gaines and Davidson (*3*) have shown that in so far as yield is affected by percentage fat content, the milk yield is inversely proportional to the energy value of the milk solids per unit milk and may be expressed as: $\frac{a}{2.66+t}$, in which a is a constant, affected in value only by factors other than fat percentage. The maintenance requirements per pound of milk, N_M, are therefore

$$\left(\frac{K_m}{\frac{a}{2.66+t}}\right)=\frac{K_m\,(2.66+t)}{a}$$

and

$$N_M=K_M\,(2.66+t)$$

where K_M is a constant $\left(\frac{K_m}{a}\right)$ *so far as affected by fat percentage.*

Since size is independent of fat percentage, it follows that growth is likewise independent of fat percentage; hence the inclusion of growth requirements in the maintenance requirements involves no error from the present standpoint. The same reasoning may be applied to fœtal growth. Obviously, however, when we include different breeds there may be some correlation (presumably negative) between fat percentage and size. If the lower testing cow is a larger cow this will imply increased maintenance and increased yield, both of which are factors in the maintenance requirements per pound of milk. The point is further considered below.

THE MILK PRODUCTION REQUIREMENTS

The total nutrients, N_T, required for milk production are the sum of the lactation and maintenance requirements. That is, $N_T=K_L\,(2.66+t)+K_M\,(2.66+t)=K_T\,(2.66+t)$.

As an intrabreed relation, then, the nutrients required for milk production, *so far as affected by fat percentage*, are K_T, $(2.66+t)$ where K_T is a constant (K_L+K_M). (The value of K_T is, of course, greatly affected by factors other than fat percentage.)

As an interbreed proposition there may be a relation between size and fat percentage such as to result in a difference in the value of the constant K_T for the different breeds. An increase in maintenance requirements tends to increase the value of K_M and an increase in production tends to decrease the value of K_M. Since the maintenance requirements vary directly as the weight of the cow, it follows that the value of K_M, and hence also the value of K_T, will vary unless production [milk yield $\times$ $(2.66+t)$] varies also directly as the weight of the cow. The relation between size of cow and production, as between different breeds, has not been adequately determined.[5] As a practical way of considering the matter, however, we may calculate the values of K_T for Holstein cows and Jersey cows, as representing the extremes in size and percentage fat content of milk among our common dairy breeds, and note how the values of K_T for these two breeds compare.

Table IV gives the values of K_T calculated on the basis of Haecker's quantitative relations as,

$$K_T=\frac{0.049(2.66+t)\text{milk yield}+(0.00792\times 365\times\text{weight})}{\text{milk yield}\,(2.66+t)}$$

[5] Various studies of the relation between size of cow and yield indicate clearly that production varies with size. Brody, Ragsdale, and Turner (*1*), from the yearly official test records of Jersey cows found that the relation between size and fat yield is expressed by the general equation, $F=aW+b$, in which F is fat yield, in pounds, W is weight of cow, in pounds, and a and b are constants. Accordingly, the nutrients for maintenance per pound of fat yielded are $\frac{2.89\,W}{aW+b}$ and increase with increasing value of W; that is, the smaller cow is more efficient than the larger. Where age is held constant a has a value near 0.2 and b near 250, and the variation in nutrients for maintenance per pound of fat, with variations in weight of the cow, is of considerable magnitude. When cows of ages 2 to 9 years are included the following equation, derived from their Table II and using their notation, describes the relation: $F=0.373\,W+104$.

Pearson (unpublished data, Illinois Experiment Station) in a study of the yearly records of 642 grade Holstein cows, unselected as to age, found a positive correlation between weight and milk yield, $r=0.304\pm0.024$. The regression line is linear, and the mean milk yields of the several weight classes are closely expressed as 6.15 times the weight. The mean fat percentage was 3.42 ± 0.01 and since fat percentage is independent of weight, Pearson's data may be translated in terms of fat, using the above notation as: $F=0.210\,W$. From the present standpoint this equation differs fundamentally from the one above for Jersey cows. Whether the difference is a matter of breed (heredity) or environment can not be said.

TABLE IV.—*Calculated values of K_T for Holsteins and Jerseys from cow-testing association and advanced registry records*

Records	Number of records	Milk	Fat	Weight	Pounds nutrients for—			K_T
					Lactation	Maintenance	Total	
		Pounds	*Per cent*	*Pounds*				
Holstein C. T. A	2,773	7,218	3.54	1,038	2,193	3,001	5,194	0.116
Jersey C. T. A	970	5,364	5.14	925	2,050	2,674	4,724	.113
Holstein A. R	5,266	14,887	3.41	1,150	4,428	3,324	7,752	.086
Jersey R. M	8,038	8,005	5.41	861	3,165	2,489	5,654	.087

The milk yields and fat percentages are from data by Gaines and Davidson (*3, p. 619*); the weights for the advanced registry records are from Table III; the weight for the Holstein C. T. A. records is the average of 793 cows included in the total (2,773); and the weight for the Jersey C. T. A. records is an estimate based on personal observation of the cows concerned.

It will be noted from Table IV that the values of K_T for Holstein and Jersey cows from the cow-testing association records are very nearly the same, 0.116 and 0.113, respectively. The value of the constant, K_T, corresponds to the theoretical pounds of nutrients required per unit of energy value (49.64 large calories) of the milk solids. K_T is virtually an inverse index of cow efficiency. The breeding and conditions of management of the cows concerned here are quite comparable. A large proportion (about 90 per cent) of the cows were high grades and the balance purebreds. The feeding and management were those of good commercial practice in the whole-milk districts of Illinois. It is quite probable, as will be shown later, that the calculated value of K_T under these conditions is very close to the actual value.

Compared with the records of the cow-testing association, the advanced registry records show a lower calculated value for K_T, namely, 0.086 for the Holstein and 0.087 for the Jerseys. But, again, under the conditions of official testing, the values for the two breeds are practically equal. The lower value of K_T is due to the higher level of production. Since many of the records included here are the result of deferred breeding and continuous milking throughout the year as well as extravagant feeding, the indicated values of K_T do not represent practical commercial milk production.

The point of immediate interest, however, is whether the relation $N_T = K_T (2.66+t)$ holds as between breeds. While the evidence is not as precise as may be desired, it does indicate so far as it goes that the relation holds reasonably close between breeds.

FEED COST IN DOLLARS

Feed cost in nutrients may be translated into feed cost in dollars by determining the price of nutrients in the feeds used. There is very little difference in the character of the ration required for milk production so far as affected by fat percentage of the milk. The lower-testing cow requires a slightly greater proportion of protein but that the effect of this difference on cost per pound of nutrients is quite negligible may be inferred from data of Ross, Hall, and Rhode (*7*). They show the cost per pound of nutrients (pasture not included) to be nearly constant so far as affected by yearly fat yield of the cow. Thus, as the yield of fat increased from 161 to 361 pounds, the cost per pound of nutrients in the feed consumed increased by less than 3 per cent, as an average of the five years 1908 to 1912.

From this it seems safe to assume that the cost per pound of nutrients is practically unaffected by the fat percentage of the milk. Hence, feed cost in dollars is proportional to feed cost in nutrients, and *so far as affected by percentage fat content of the milk*

$$FC = K\ (2.66+t)$$

in which FC is feed cost ($) per pound of milk; t is per cent of fat in the milk; and K is a constant dependent in value on the price level of feeds and on the value of K_T.

While the point under consideration here is *relative* costs, rather than *absolute* costs, it is perhaps worth while to draw a comparison as to absolute feed cost. Pearson (*6*), by cost accounting methods, found the cow feed cost of milk production to be $1.05 per hundredweight of milk for

the years 1914–1916, in the Illinois Chicago district. The average fat test was 3.56 per cent. The conditions of production were quite similar and entirely comparable to the conditions of the Holstein cow testing associations referred to in Table IV. Using the data of Ross for 1914–1916 the average cost of digestible nutrients was $0.0153 per pound. This figure may be taken to represent the price level of feeds at the time of Pearson's investigation and on this basis $K = \$0.0153\ K_T$, or, taking K_T from Table IV, $K = \$0.0153 \times 0.116 = \$0.00177$ Substituting these values in the feed cost equation we have $FC = \$0.00177\ (2.66 + 3.56) = \$0.011$. That is, the feed cost calculated by the equation is $1.10 per hundredweight, corresponding quite closely to Pearson's cost-accounting data, $1.05 per hundredweight. Pearson's silage evaluation is lower than that used by Ross. There is also the question as to the cost of nutrients from pasture in Pearson's data (pasture is not included in Ross's data). On the whole, the feed-cost equation above may be regarded as in accord with Pearson's cost-accounting data.

COSTS OTHER THAN FEED

The data on feed costs as interpreted above are, to the writer's mind, quite conclusive. The costs other than feed constitute a separate problem to be solved by cost-accounting methods rather than by biological methods.[6] Until the economists help us out in the matter it may be assumed tentatively without any apparent gross error that the other costs are roughly proportional to feed costs. This assumption does not imply that there is a constant ratio of feed cost to total cost, but merely that under conditions where, for example, feed cost is 60 per cent of the total cost of milk production for the 3 per cent cow it will be 60 per cent also for the 4 per cent cow under the same conditions. As between breeds, especially, there are a number of cost factors directly or indirectly involved, such as cost of milk hauling, beef value of cow, veal value of calves, etc., but these are all minor items. If we may assume that the total cost of milk production is proportional to feed cost, *so far as affected by fat percentage*, it is possible to make some comparisons with reference to the price differential for fat percentage in whole-milk markets.

THE DIFFERENTIAL IN WHOLE-MILK PRICES

The great bulk of the whole-milk market is for city supply. Judging by the August, 1923, "Fluid Milk Market Report for the United States" of the United States Department of Agriculture,[7] the markets of about 11 per cent of our cities still retain the flat-rate basis of payment, while 89 per cent make some sort of distinction or differential in price according to the fat test of the milk. Both the producer and the buyer are interested in this connection in the effect of fat percentage on cost of production. The buyer's price is an expression of demand, and the producer seeks to adjust his production to that demand, as expressed in price, which offers the largest profit. If the buyer wishes milk of a certain fat test he will discriminate in price against milk of another fat test out of proportion to the cost of production. On the other hand, if he wishes to encourage equally the production of milk of various fat tests he will pay in proportion to the cost of production, as affected by percentage of fat content.

According to the equation developed above, the cost of production, *so far as affected by fat percentage*, may be expressed as a function of the fat percentage, and likewise the difference in cost of production, corrresponding to a certain difference in fat percentage, may be expressed as a function of the cost of production of milk of any definite or base fat percentage. If we take 3.5 as the base fat percentage and a "point" or 0.1 as the difference in fat percentage, then the difference in cost per point is $\left(\frac{0.1}{2.66+3.5}\right) C_{3.5}$, where $C_{3.5}$ is the cost of 3.5 per cent milk. That is, if the price differential is proportional to the cost differential it will be 1.6234 per cent of the price of 3.5 per cent milk. In like manner, it will be 1.7668 per cent of the price of 3 per cent milk; 1.5015 per cent of 4 per cent milk; or 1.3055 per cent of 5 per

[6] Some may regard the whole matter as a problem for the cost accountant alone. One can scarcely doubt, however, that the effect of fat percentage on cost is a material factor in the dairyman's profit, and has been especially so in the past when the flat rate for whole milk was so generally in effect. But for some reason the economist seems to have passed by this item in his rather detailed analyses of farm management and profits.

[7] United States Department of Agriculture, Bureau of Agricultural Economics, Market News Service. Fluid milk market report for the United States, August, 1923. 4 p. 1923 [Mimeographed].

cent milk. A price differential greater than that indicated is favorable to the production of high-testing milk; while a smaller differential is favorable to the production of low-testing milk.

Obviously, the price differential should be considered relative to the price of milk. Figure 2 shows in the form of a frequency distribution curve the differentials for 101 cities taken from the August, 1923, market report above referred to.

The differential is expressed as a per cent of the price[8] of 3.5 per cent milk, and all cities are included where a definite price and differential are given. For the sake of comparison the cost of production differential is indicated in the figure, expressed likewise as a per cent of the cost of 3.5 per cent milk. Except for the two classes, 0 and 2.8, there appears to be a tendency toward a normal distribution curve centering about the cost of production as a mean. The class at 0 represents those cities paying a flat rate. The class at 2.8 represents those cities paying, practically, on a straight fat basis (a differntial of 2.857 per cent of the price of 3.5 per cent milk is a straight fat basis).

An exact adjustment of price in proportion to cost is rather cumbersome by the system of a certain allowance per 0.1 per cent in the fat test. It may be more simply accomplished by adjusting the price according to one of the following formulæ, in which t stands for fat percentage and $P_{3.0}$, the price of 3.0 per cent milk; $P_{3.5}$, the price of 3.5 per cent milk; etc.:

$0.17668\ P_{3.0}\ (2.66+t)$

$.16234\ P_{3.5}\ (2.66+t)$

$.15015\ P_{4.0}\ (2.66+t)$

$.13055\ P_{5.0}\ (2.66+t)$ or, generalized,

$$\left(\frac{1}{2.66+b}\right)(P_b)\ (2.66+t)$$

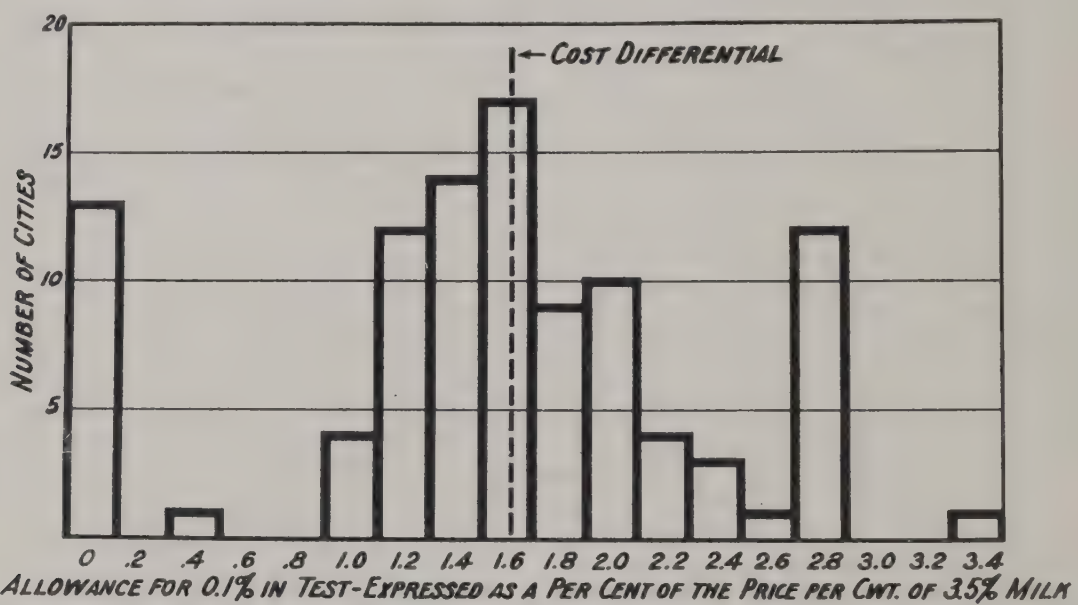
20
15
10
5
NUMBER OF CITIES
← COST DIFFERENTIAL
0 .2 .4 .6 .8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4
ALLOWANCE FOR 0.1% IN TEST-EXPRESSED AS A PER CENT OF THE PRICE PER CWT. OF 3.5% MILK

FIG. 2.—Frequency distribution curve of 101 cities with respect to the differential in the price of milk according to the fat test of the milk. Data from August, 1923, "Milk Market Report." The differential is the price difference per hundredweight of milk for each difference of 0.1 in the per cent of fat in the milk. In order to make the data comparable it is expressed, in this figure, as a per cent of the price per hundredweight of 3.5 per cent milk. The broken line at 1.6 indicates the cost of production differential; that is, the difference in cost of production per hundredweight of milk for each difference of 0.1 in the per cent of fat in the milk (so far as the cost of milk production is affected by percentage fat content of the milk). The cost differential is expressed, likewise, as a per cent of the cost of production of 3.5 per cent milk. It is assumed that the cost of production is proportional to the feed cost

To illustrate the working of the above scheme, we may take the Chicago market for August, 1923, \$3.20 per hundredweight for 3.5 per cent milk. The price for milks of other test, to bear the same ratio to cost of production, would be $\$0.16234 \times 3.20\ (2.66+t)$ and $\$0.52\ (2.66+t)$ would be the general expression for the price of milk for that month. Thus, 3.21 per cent milk would be $\$0.52\ (2.66+3.21) = \$3.05$; 4.14 per cent milk would be $\$0.52\ (2.66+4.14) = \$3.54$; etc. The prices actually paid were \$3.08 and \$3.46, respectively.

SUMMARY

The nutrients required for lactation per pound of milk, N_L, may be expressed as a function of the fat per-

[8] This price is f. o. b. city. It would be better for this comparison to use country buying prices and weighted average for the year. The data for such a comparison are not available to the writer.

centage of the milk, t, as: $N_L = K_L (2.66+t)$. The nutrients required for maintenance per pound of milk, N_M, (because of the nature of the relation existing between fat percentage and size of cow and between fat percentage and milk yield) may also be expressed as a function of fat percentage, as: $N_M = K_M (2.66+t)$. Consequently the total nutrients required for milk production per pound of milk, N_T, may be expressed as: $N_T = K_T (2.66+t)$. The cost in dollars per pound of nutrients is practically unaffected by fat percentage and consequently the feed cost ($) per pound of milk, FC, may be expressed, as: $FC = K (2.66+t)$. In these relations K_L, K_M, K_T, and K are constants *so far as affected by fat percentage.*

The price differential for fat test as judged by the whole-milk markets of 101 cities seems to be tending to distribute about a mean which is approximately the same as the cost of production differential. The price of milk may be adjusted in proportion to the cost of production by the equation $P = \frac{P_b}{2.66+b} (2.66+t)$, in which P_b is the base price, b is the fat percentage to which the base price applies, and P is the price to be paid according to the fat test, t, of the milk delivered. This assumes that the cost of production is proportional to the feed cost of milk production, *so far as affected by fat percentage.*

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RELATION BETWEEN THE DIET, THE COMPOSITION OF THE BLOOD, AND THE SECRETION OF MILK OF DAIRY COWS[1]

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INTRODUCTION

It has been shown that with milking cows the lactose, fat, and proteins of milk are made in the mammary gland from glucose (*10*),[2] phosphatide (*11*), and amino acids (*3*), respectively, which are taken directly from the plasma of the blood. Meigs (*12*) has pointed out that within the limits of the experimental errors involved the mammary gland takes these precursors out of the plasma in approximately the proportion in which the corresponding constituents occur in the milk. This, as he points out, furnishes strong evidence for the view that the lactose, fat, and proteins of milk are, respectively, derived entirely from the dextrose, phosphatide, and free amino acids of the blood.

Abrupt changes in the rations of milking cows frequently produce decided changes in the yield and composition of milk. The concentration of some of the milk precursors in the blood plasma may be determined with a fair degree of accuracy. A study of the composition of the rations, blood, and milk simultaneously might, therefore, be expected to throw much light on important problems in the physiology of milk secretion. It would also throw light upon the relation between the diet and the composition of the blood with animals where the process of absorption on a given ration is practically constant. The cow furnishes an excellent subject in many respects for such a study.

The plasma amino N in milking cows is low (*2*, *3*). This is especially true when they are fed according to the ordinary standards of feeding. It is seldom so high as 3 mg. per 100 c. c. On an average, 25 per cent of this is taken out in a single passage of the blood through the gland. This means a very great depletion of some of the individual amino acids. It would seem likely, a priori, that the yield and composition of milk might, therefore, frequently be affected and limited by changes in either the concentration or composition of this amino-acid mixture. Hence, especial attention in this study has been given to the relation of the amino acids of the blood to the diet and the secretion of milk.

In the experiments to be reported milking cows were used. In their rations the protein was varied in amount or quality or both, and the energy content was varied either alone or together with the amount of protein. The amino N of the blood and plasma was determined at intervals. In some experiments it was found possible to follow simultaneously the amino N and the concentration of a particular amino acid (tryptophane) in the blood and thus to get some idea of the qualitative changes occurring in the blood mixture of amino acids. The glucose and lipoid P of the blood and plasma were determined in some experiments. The yield of milk and its content of N and fat were followed throughout. The milk lactose was determined in some experiments.

EXPERIMENTAL ROUTINE

The experiments, with the exception of No. VI, were begun with cows on rations which were adequate, or nearly adequate, in both protein and energy, according to the commonly accepted feeding standards for maintenance and milk yield (*7*, *p. 133*). The initial periods on approximately adequate rations were followed by periods in which the rations were made markedly inadequate in either energy or protein content, or both, the protein mixture in the ration being changed in either quantity or composition, or both. These periods of inadequate feeding were generally followed by periods in which the adequacy of the rations was again restored. The changes in rations were made abruptly and without transition periods.

Except for the hay, the feed was given in two equal portions, one between 4 and 5 a. m. and the other between 1 and 2 p. m. The daily allowance of hay was fed at the afternoon feeding. Each cow was milked at the same time daily (4.30 to 4.45

[1] Received for publication May 12, 1924—issued March, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 624.

a. m. and at 3.15 to 3.45 p. m.); and, so far as possible, the milking was done by the same man throughout each experiment. The blood samples were taken at 8.30 to 9 a. m.

In all the experiments except Nos. I and II the Ca, P, and Na contents of the rations were kept constant by adding varying amounts of ground limestone, $Na_2HPO_4.12H_2O$, and NaCl, as shown in Table II. The calculations of the amounts of these salts to be added were based on determinations found in the literature or made at various times in this laboratory. The cows also had access to NaCl when turned out daily in a bare lot.

ANALYTICAL METHODS

The samples of blood in Experiments I, II, III, V, and VIII were 450 to 1,000 c. c. They were collected in bottles containing 0.1 gm. of sodium oxalate per 100 c. c. of sample. In Experiments IV, VI, and VII the samples of blood were larger; and 0.7 c. c. of a solution of sodium citrate (38 gm. per 100 c. c. of solution) was used per 100 c. c. blood. The plasma was immediately separated by centrifuging 20 minutes at 3,600 revolutions per minute. The analytical samples for the blood and plasma determinations were taken out as quickly as possible.

The lipoid phosphorus of the blood plasma was calculated as the difference between the total and the inorganic phosphorus in the plasma as determined according to the methods described by Meigs, Blatherwick, and Cary (*11*). The amino acid N of the blood and plasma was determined as previously described (*3*) except that, beginning with the sample of July 22, Experiment I, the urease was reduced to one-tenth that previously used. The determinations on the Van Slyke apparatus were made at the same room temperature throughout each experiment. The reducing sugar of the blood and plasma was determined by the revised method of Folin and Wu (*6*). The blood corpuscle volume was measured off on a marked centrifuge tube.

The p-dimethylamidobenzaldehyde reagent of Herzfeld (*8*) was used in the determination of the tryptophane of the blood and blood plasma. The protein was precipitated either as in the amino N determination (cow 246), or by coagulation with acetic acid as in the amino N determination and subsequent precipitation with aldehyde-free alcohol[3] (cow 423). The tryptophane in the concentrated protein-free extracts was precipitated in the presence of 7 per cent (by volume) of sulphuric acid and 20 per cent of mercuric sulphate. The mercury-tryptophane precipitate was redissolved in H_2O and 5 per cent NaCN (total volume=5 c. c.). Then 2 c. c. of the reagent and 23 c. c. of hydrochloric acid were added. With cow 246 hydrochloric acid that had been saturated at 0° C. was used. With cow 423 ordinary hydrochloric acid was used and then HCl gas was run in slowly for 10 minutes, while cooling in tap water. They were then heated at about 60° C. in a water bath until the color was fully developed (30 minutes). They were compared with tryptophane standards similarly treated with the Herzfeld reagent, HCl, etc. Generally, three or more standards were run, because, although they usually agreed well, it was found that sometimes they did not. This method of determining the protein-free tryptophane is not entirely satisfactory; the colors do not match well. In Experiments IV and VI the unknowns and standards were diluted 1:2 with water before reading. The writers believe that the data for free tryptophane obtained by them are sufficiently reliable to warrant publication and the conclusions that they have drawn from them. Further work is in progress upon methods of determining free tryptophane in blood and plasma. The results of these investigations will be published later.

The milk was preserved by adding to each 100 c. c. either 10 drops of chloroform (Experiment I), 2 c. c. of formalin (Experiments II, III, V, and VII), or 2 c. c. of a mixture of 50 gm. of phenol and 10 c. c. of 95 per cent alcohol (Experiments IV, VI, and VII). The nitrogen in the milk was determined by the Kjeldahl-Gunning-Arnold (*1*) method, the fat by the ordinary Babcock method, and the lactose by means of a polariscope. The proteins in the lactose determinations were precipitated by acid mercuric nitrate according to the "Methods of Analysis" of the Association of Official Agricultural Chemists (*1*).

The analytical data shown in the tables are generally averages of two or more determinations except where lactose was used, in which case only single determinations were made. The tryptophane determinations in Experiment VIII were not run in duplicate.

The changes made in the rations and the effects thereby brought about on the composition of the blood and milk and on the quantity of milk yielded are given in detail in Tables I to X.

[3] Refluxed and distilled off from KOH.

TABLE I.—*General schedule of experiments*

Period No.	Dates		Digestible feed [a] daily					Remarks
			Offered [b]			Feed offered, as percentage of feed required [c]		
	From—	To—	Protein	Nutrients (total)	Fat	Protein	Nutrients	
			Kg.	*Kg.*	*Kg.*			
EXPERIMENT I.—COW 17. ENERGY AND PROTEIN CONTENT OF RATION REDUCED								
1	May 1, 1920	May 11, 1920, a. m.	0.864	6.21	0.321	82.4	85.8	Nearly adequate ration.
2	May 11, 1920, p. m.	June 7, 1920, a. m.	.494	4.11	.201			Energy and protein reduced in amount, no change in quality of protein.
3	June 7, 1920, p. m.	June 24, 1920	.864	6.21	.321			Nearly adequate ration.
EXPERIMENT II.—COW 54. ENERGY OF RATION REDUCED								
1	Nov. 25, 1920	Nov. 27, 1920	1.037	7.34	0.231	98.2	95.8	Nearly adequate ration.
2	Nov. 28, 1920	Dec. 24, 1920	1.036	4.89	.159			Energy of ration reduced, no change in protein.
3	Dec. 25, 1920	Jan. 15, 1921	1.037	7.34	.231			Nearly adequate ration.
EXPERIMENT III.—COW 422. PROTEIN REDUCED IN QUANTITY, NO CHANGE IN QUALITY								
1	Sept. 16, 1921	Sept. 21, 1921	0.902	6.59	0.299	103.7	102.2	Nearly adequate ration.
2	Sept. 22, 1921	Oct. 10, 1921	.471	6.63	.222			Protein reduced in quantity, no change in quality.
3	Oct. 11, 1921	Oct. 29, 1921	.902	6.59	.299			Nearly adequate ration.
EXPERIMENT IV.—COW 246. PROTEIN REDUCED IN QUANTITY, NO CHANGE IN QUALITY								
1	Oct. 27, 1922	Nov. 4, 1922, a. m.	1.225	7.29	0.277	92.1	88.8	Nearly adequate ration.
2	Nov. 4, 1922, p. m.	Dec. 9, 1922	.620	7.25	.257			Portein reduced in quantity, no change in quality.

[a] The digestible constituents of the feed are calculated from tables given by Henry and Morrison (*7 Appendix, Table III*).

[b] Feed offered was completely consumed in Experiments I, II, IV, V, and VIII. The feed refused in Experiments III, VI, and VII is indicated in footnotes to Tables V, VIII, and IX, respectively.

[c] Requirements based on Savage standard for feeding of milking cows (*7 p. 133*).

TABLE I.—*General schedule of experiments*—Continued

Period No.	Dates		Digestible feed daily					Remarks
			Offered			Feed offered, as percentage of feed required		
	From—	To—	Protein	Nutrients (total)	Fat	Protein	Nutrients	
			Kg.	*Kg.*	*Kg.*			
EXPERIMENT V.—COW 54. PROTEIN REDUCED IN QUANTITY AND QUALITY								
1	Oct. 22, 1920	Oct. 28, 1920	1.037	7.34	0.231	91.0	90.7	Nearly adequate ration containing casein and lactalbumin.
2	Oct. 29, 1920	Nov. 13, 1920	.514	7.34	.231			Sucrose substituted for the casein and lactalbumin.
3	Nov 14, 1920	Nov. 27, 1920	1.037	7.34	.231			Nearly adequate ration containing casein and lactalbumin.
EXPERIMENT VI.—COW 246. QUANTITY OF PROTEIN INCREASED, QUALITY IMPROVED								
1	Dec. 2, 1922	Dec. 9, 1922	0.620	7.25	0.257	63.8	98.9	Ration deficient in protein.
2	Dec. 10, 1922	Dec. 22, 1922	1.195	7.25	.257			Casein put in ration in place of dextrine.
3	Dec. 23, 1922	Feb. 21, 1923	1.468	7.29	.246			Further addition of casein.
4	Feb. 22, 1923	Mar. 13, 1923	1.321	7.88				Cow put on ordinary grain mixture, silage, and alfalfa.
EXPERIMENT VII.—COW 246. CHANGE IN QUALITY OF PROTEIN, NO CHANGE IN QUANTITY								
1	Sept. 4, 1922	Sept. 13, 1922	1.230	7.27	0.209	97.3	83.6	Casein-corn-protein ration.
2	Sept. 14, 1922	Sept. 28, 1922	1.225	7.29	.277			Corn-protein ration.
EXPERIMENT VIII.—COW 423. CHANGE IN QUALITY OF PROTEIN, NO CHANGE IN QUANTITY								
1	Nov. 5, 1921	Nov. 12, 1921	0.775	5.67	0.229	103.3	100.2	Corn-protein ration.
2	Nov. 13, 1921	Nov. 23, 1921	.775	5.66	.134			Gelatin-corn-protein ration.
3	Nov. 24, 1921	Dec. 5, 1921	.775	5.66	.134			Milk-protein and corn-protein ration.

17642—24†——3

TABLE II.—*Quantities of separate feeds offered daily* [a]

EXPERIMENT I.—COW 17. (WEIGHT 375 KG.)

Period No.	Corn starch; corn cellulose	Sucrose	Casein	Lactalbumin	Gelatin	Corn meal	Corn gluten feed	Cottonseed meal	Linseed meal	Hominy meal	Wheat bran	Ground oats	Alfalfa hay	Timothy hay	Corn silage	$CaCO_3$	$NaH_2PO_4.12H_2O$	NaCl	Remarks
	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	Kg.	
1								0.292	0.292	1.169	0.780	1.949	1.81		11.34			0.059	Adequate ration.
2								.146	.146	.585	.390	.974	.91		11.34			.029	Energy and protein reduced.
3								.292	.292	1.169	.780	1.949	1.81		11.34			.059	Adequate ration.

EXPERIMENT II.—COW 54. (WEIGHT 472 KG.)

1	0.769		0.448	0.075		2.55					1.09			2.72	10.89			0.051	Adequate ration.
2			.590	.098		1.61					.69			2.04	8.16			.051	Energy reduced.
3	.769		.448	.075		2.55					1.09			2.72	10.89			.051	Adequate ration.

EXPERIMENT III.—COW 422. (WEIGHT 334 KG.)

1						3.953	1.438	0.450	0.225						9.07	0.091	0.354	0.067	Adequate ration.
2	1.132	0.453				3.800		.226	.113						9.07	.098	.531	.067	Quantity of protein reduced.
3						3.953	1.438	.450	.225						9.07	.091	.354	.067	Adequate ration.

EXPERIMENT IV.—COW 246. (WEIGHT 610 KG.)

1						0.33	4.62							1.81	13.61	0.049	0.360	0.176	Adequate ration.
2	0.575					3.17	.91							1.81	13.61	.058	.637	.188	Quantity of protein reduced.

[a] All except the hay and silage were fed as a concentrate mixture.

TABLE II.—*Quantities of separate feeds offered daily* [a]—Continued

EXPERIMENT V.—COW 54. (WEIGHT 484 KG.)

Period No.	Corn starch; corn dextrine	Sucrose	Casein	Lactabumin	Gelatin	Corn meal	Corn gluten feed	Cottonseed meal	Linseed meal	Hominy meal	Wheat bran	Ground oats	Alfalfa hay	Timothy hay	Corn silage	$CaCO_3$	$Na_2HPO_4.12H_2O$	NaCl	Remarks
	Kg.	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	*Kg.*	
1	0.769	------	0.448	0.075	------	2.55	------	------	------	------	1.09	------	------	2.72	10.89	------	------	0.051	Adequate ration.
2	.769	0.523	------	------	------	2.55	------	------	------	------	1.09	------	------	2.72	10.89	------	------	.051	Quantity and quality of protein reduced.
3	.769	------	.448	.075	------	2.55	------	------	------	------	1.09	------	------	2.72	10.89	------	------	.051	Adequate ration.

EXPERIMENT VI.—COW 246. (WEIGHT 596 KG.)

1	0.575	------	------	------	------	3.17	0.91	------	------	------	------	------	------	1.81	13.61	0.058	0.637	0.188	Protein inadequate.
2	------	------	0.575	------	------	3.17	.91	------	------	------	------	------	------	1.81	13.61	.058	.637	.188	Protein increased in quantity and quality.
3	------	------	.869	------	------	2.86	.91	------	------	------	------	------	------	1.81	13.61	.059	.642	.190	Further increase in protein.
4	------	------	------	------	------	2.16	------	1.078	0.539	------	1.62	------	2.72	------	13.61	------	------	.054	Ordinary ration.

EXPERIMENT VII.—COW 246. (WEIGHT 582 KG.)

1	1.140	------	0.565	------	------	0.97	1.82	------	------	------	------	------	------	1.81	13.61	0.057	0.596	0.187	Casein-corn-protein ration.
2	------	------	------	------	------	.33	4.62	------	------	------	------	------	------	1.81	13.61	.049	.360	.176	Corn-protein ration.

EXPERIMENT VIII.—COW 423. (WEIGHT 307 KG.)

1	------	------	------	------	------	2.65	2.28	------	------	------	------	------	------	------	9.07	0.073	0.230	0.056	Corn-protein ration.
2	1.480	0.456	------	------	3.88	1.13	.97	------	------	------	------	------	------	------	9.07	.076	.395	.061	Gelatin-corn-protein ration.
3	1.480	.456	0.320	0.068	------	1.13	.97	------	------	------	------	------	------	------	9.07	.076	.395	.061	Milk-corn-protein ration.

[a] All except the hay and silage were fed as a concentrate mixture.

DISCUSSION

It will be convenient to consider the data in these experiments in the following order:

1. Changes in the composition of the blood produced by the changes in the rations.
2. Changes in the yield and composition of the milk produced by the changes in the rations.
3. Physiological relations between the composition of the blood and the secretion of milk.

CHANGES IN THE COMPOSITION OF THE BLOOD

An inspection of Tables III to X will readily give some idea of the concentrations of plasma amino N that may occur under normal conditions. There are 11 determinations in these experiments made with cows that had been fed ordinary feeding materials, containing approximately the quantity of protein, and from 85 to 102 per cent of the energy, which would be required according to the Savage standard, the feeding being continued for a sufficient length of time to give an idea of the normal plasma amino N under these conditions. The figures varied from 2.20 to 2.64 mg. per 100 c. c. of blood plasma.

Figure 1 shows the changes in plasma amino N that occurred as a result of

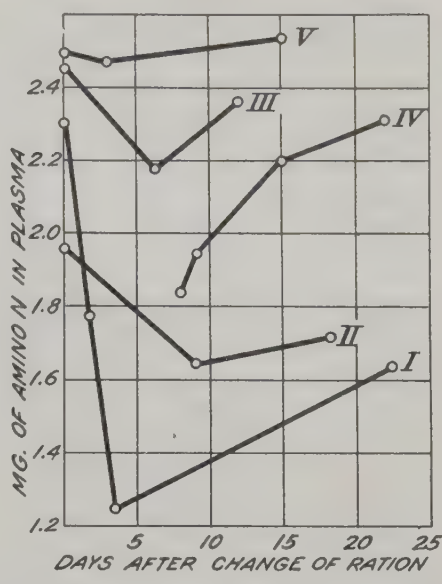

FIG. 1.—Effect on plasma amino N of reducing the protein (50 per cent) or energy content (33 per cent), or both, in the ration of a milking cow after a period on an adequate ration. Except in Experiment IV, the initial determinations give the level of plasma amino N in the first periods just before the rations were changed

Experiment I.—Energy and protein content of ration reduced

Experiment II.—Energy content of ration reduced

Experiment III.—Amount of protein reduced; no change in quality

Experiment IV.—Amount of protein reduced; no change in quality. No amino N determinations were made before the change of ration. This curve, like the others, shows a tendency for concentration of amino N to return to normal even while ration is still inadequate

Experiment V.—Quantity and quality of protein reduced

TABLE III.—*Experiment I, cow 17. Results of changing the energy content and the quantity of protein in the ration*

Period No.	Date	Milk (daily)					Blood		
		Yield	Nitrogen		Fat		Amino N[a]		Plasma lipoid phosphorus (mg. per 100 c. c.)
							Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	
	1920	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*			
1	May 1–10	12.85							
1	May 11	12.93	0.5045	65.23	3.43	443.9	4.10	2.30	7.8
2	May 12	12.25	.4922	60.28					
2	May 13	10.71	.5211	55.78	4.04	432.8	3.74	1.77	
2	May 14	9.89	.4964	49.08	3.88	384.1			
2	May 15	9.89	.4911	48.56	3.93	388.7	3.23	1.25	7.0
2	May 16–18	9.89							
2	May 19	10.30	.4676	48.15	3.48	358.7			
2	May 20–27	8.84							
2	May 28	9.21	.4738	43.63	3.32	304.1			
2	May 29–June 1	8.47							
2	June 2	7.94	.4600	36.30	3.62	284.3			
2	June 3	8.16					3.03	1.63	
2	June 4–6	7.76							
2	June 7	7.80	.4848	37.83	3.61	281.4	3.05	1.66	6.3
3	June 8	8.26							
3	June 9	10.84	.5292	57.13	3.81	423.3			
3	June 10	9.43					2.75	1.57	
3	June 11	9.30			3.94	366.4			
3	June 12–23	9.25							
3	June 24	9.03					3.63	2.64	

[a] Average difference in duplicate amino-N determinations, in blood 9.2 per cent, and in plasma 9.8 per cent.

reductions in these rations in the second periods of Experiments I to V. The curve in Experiment I shows that a sharp reduction in both protein and energy content of the ration of a milking cow produces a decided and abrupt drop in plasma amino N. In four days it dropped from 2.3 to 1.25 mg. per 100 c. c., or 46 per cent.

Experiment II shows that a similar drop in plasma amino N occurs when the energy content of the ration is reduced without materially altering the dietary protein. In period 1 of this experiment the cow was just recovering from the effects of a previous experiment, and the plasma amino N of 1.95 mg. per 100 c. c. was low. The energy content of the ration (Table I) was relatively higher than in Experiment I. Yet the plasma amino N dropped to 1.64 mg. per 100 c. c., or 16 per cent.

In Experiment II determinations were made of the glucose in the blood and plasma in order to determine whether this blood constituent underwent changes parallel to the changes in animo N. The results indicate that this was not the case; apparently the sugar content of the blood samples was more affected by the disturbance caused to the animal in obtaining them than by the changes in the diet. (See Table IV and its footnotes.)

TABLE IV.—*Experiment II, cow 54. Change in energy content of ration*

Period No.	Date	Milk (daily)							Blood			
		Yield	Nitrogen		Fat		Lactose		Amino N [a]		Sugar	
									Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1920	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*				
1	Nov. 25	11.66										
1	Nov. 26	11.34	0.4837	54.85	3.44	390.1	5.23	593.1				
1	Nov. 27	11.84	.4946	58.55	3.64	430.9	5.34	632.2	4.05	1.95	64.1	77.1
2	Nov. 28	11.11										
2	Nov. 29	11.34	.4598	52.14	3.77	417.5	5.39	611.2				
2	Nov. 30	10.52										
2	Dec. 1	11.16	.4539	50.65	3.39	378.3	5.42	628.2				
2	Dec. 2	11.16	.4714	52.68	3.37	376.1						
2	Dec. 3–4	11.09										
2	Dec. 5	10.71	.4592	49.16	3.49	373.6	5.37	574.9				
2	Dec. 6	10.52	.4661	49.05	3.37	354.7	5.39	567.2				
2	Dec. 7	10.93							3.87	1.64	63.5	70.9
2	Dec. 8	10.39	.4419	45.91	3.47	360.5						
2	Dec. 10	10.57	.4547	48.06	3.61	381.5						
2	Dec. 11	10.61	.4502	48.01	3.43	364.1						
2	Dec. 12	10.80	.4646	50.16	3.49	376.8						
2	Dec. 13	10.30	.4569	47.04	3.20	329.5						
2	Dec. 15	10.39	.4424	45.95	3.21	333.5						
2	Dec. 16	9.35							3.72	1.72	[b] 73.6	[b] 89.8
2	Dec. 17	9.34										
2	Dec. 18–24	9.66										
3	Dec. 25	9.33										
3	Dec. 26	9.66										
3	Dec. 27	10.61										
3	Dec. 28	10.61										
3	Dec. 29–31	10.48										
	1921											
3	Jan. 1–7	10.52										
3	Jan. 8	10.93	.4702	51.40	3.40	371.7						
3	Jan. 9	10.66			3.39	361.4						
3	Jan. 10	10.30	.4675	48.14	3.40	350.1						
3	Jan. 11	9.98			3.43	342.3						
3	Jan. 12	10.43	.4772	49.79	3.86	402.7						
3	Jan. 13	9.71			3.51	340.7						
3	Jan. 15	9.89	.4819	47.65	3.51	347.1			4.14	2.47	[c] 54.7	[c] 61.3

[a] Average difference in duplicate amino-N determinations in blood 3 per cent, and in plasma 4.3 per cent.

[b] This cow was most disturbed in obtaining this blood sample.

[c] This cow was least disturbed in obtaining this blood sample.

TABLE V.—*Experiment III, cow 422. Change in the quantity of protein in the ration; no change in its quality*

Period No.	Date	Milk (daily)					Amino N[a]	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1921	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*		
1	Sept. 16–21	8.65	5.01	433	0.643	55.6	[b] 4.22	[b] 2.45
2	Sept. 22 [c]							
2	Sept. 25	7.12	4.80	342	.613	43.7		
2	Sept. 26	6.99	4.98	348	.613	42.2		
2	Sept. 27	6.76	4.81	325	.625	42.2		
2	Sept. 28	6.12	4.74	290	.643	39.3	3.76	2.17
2	Sept. 29	6.31	4.65	293	.620	39.1		
2	Sept. 30	6.49	4.55	295	.623	40.4		
2	Oct. 1	5.85	5.38	315	.628	36.7		
2	Oct. 2	5.72	4.78	273	.600	34.3		
2	Oct. 3	5.67	5.03	285	.660	37.4		
2	Oct. 4 [c]						3.76	2.36
2	Oct. 6	5.53	5.77	320	.636	35.2		
2	Oct. 7	5.31	5.57	296	.640	34.0		
2	Oct. 8	5.44	5.30	288	.644	35.0		
2	Oct. 10 [c]							
3	Oct. 11 [c]	5.67	4.96	281	.644	36.5		
3	Oct. 12	6.40	5.03	322	.606	38.8		
3	Oct. 13	6.80	6.14	418	.643	43.7		
3	Oct. 14	6.89	4.83	333	.679	46.8	4.73	
3	Oct. 15	6.58	5.83	383	.705	46.4		
3	Oct. 16	6.99	5.94	415	.712	49.8		
3	Oct. 17	6.89	5.94	410	.722	49.8	4.36	2.33
3	Oct. 18	6.99	5.90	412	.710	49.6		
3	Oct. 19	7.21	5.77	416	.676	48.8		
3	Oct. 20	7.22	5.92	427	.682	49.2		
3	Oct. 21	7.26	5.74	417	.732	53.2		
3	Oct. 22	7.08	6.45	456				
3	Oct. 25	7.54	6.35	478	.722	54.4		
3	Oct. 26	7.12	6.25	445	.750	53.4	4.63	2.36
3	Oct. 27	6.85	6.33	433	.748	51.2		
3	Oct. 28	7.44					3.94	2.20
3	Oct. 29	7.12	6.24	444	.729	51.9		

[a] Average difference in duplicate amino-N determinations in blood 4.9 per cent, and in plasma 3.1 per cent.

[b] Sample taken Sept. 19.

[c] Feed refused or withheld: Sept. 22, grain 2.01 kg.; Oct. 4, silage 1.97 kg. and grain 1.38 kg.; Oct. 10, grain 1.1 kg.; Oct. 11, grain 3.43 kg.

In Experiment III, in which the quantity of dietary protein in the ration was reduced without change in its quality[4] or in the energy content of the ration, there was initially a slight drop in plasma amino N. In period 1 it was 2.45 mg. Six days after the change of ration it was 2.17 mg., or 11.4 per cent lower. This is hardly considerable.

A comparison of the curves in Experiments I and III shows plainly that the cut in the energy along with the protein in Experiment I increased decidedly the abruptness and magnitude of the drop in plasma amino N. From this and from Experiment II it is evident that a *sharp cut (33 per cent) in the energy content of the ration of a milking cow brings about a decided reduction in plasma amino N. The energy content of the ration is an important factor in determining the level of plasma amino N.*

The curve in Experiment V shows that no change in plasma amino N occurred when the quality as well as the quantity of the dietary protein was reduced. In period 1 the plasma amino N was 2.43 and 2.55 mg., and in period 2 it was 2.47 and 2.53 mg. This result, compared with that in Experiment III, suggests that the quality of the dietary protein may affect the level of plasma amino N. (See later.) Experiments III and V indicate that *a sharp cut of 50 per cent in the dietary protein with a milking cow often does not*

[4] By quality here we mean the amino acid composition of the mixture of dietary proteins. Wherever used with reference to proteins in this paper, it is used in this sense. By a protein of good quality we mean one that because of its amino acid composition can be used efficiently in the synthesis of milk and tissue proteins.

greatly affect the level of the plasma amino N.

In Experiment IV the amount of protein was reduced without change in its quality and without change in the energy content of the ration. No determinations of the amino N were made during the period on an adequate ration. The concentration of amino N in the blood plasma on the eighth day of the second period is decidedly lower than the average concentration of amino N found in these experiments for cows on adequate rations; but this may be partly due to the low energy content of this cow's ration. (See Table I.)

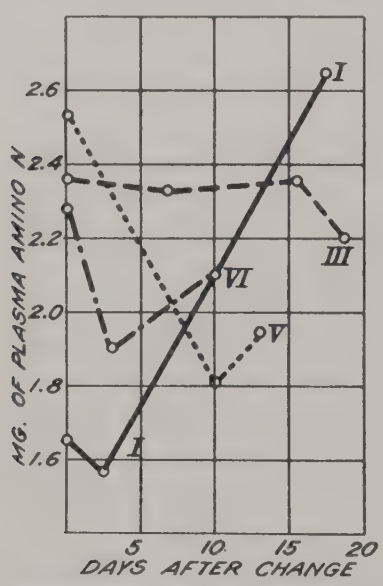

FIG. 2.—Effect on plasma amino N of an increase of 75 to 100 per cent in the amount of protein or 50 per cent in the energy content, or both, in the ration of a milking cow after a period on a ration inadequate in these constituents. The first determinations in this chart show the level of the plasma amino N just before the rations were increased

Experiment I.—Amount of energy and protein in ration increased

Experiment III.—Amount of protein increased; no change in quality

Experiment V.—Amount and quality of protein increased

Experiment VI.—Amount and quality of protein increased

NOTE.—Experiment II does not appear in this chart because no determination of amino N was made in Experiment II until 21 days after adequacy of ration was restored. (See Table V.)

After the initial drop in plasma amino N in Experiments I, III, and IV there was a subsequent rise. In Experiment I the plasma amino N on May 15 was 1.25 mg. per 100 c. c. On the same ration on June 3 and 7 it was 1.63 and 1.66 mg., respectively, or 32 per cent higher. In Experiment IV it rose from 1.84 mg. on November 13 to 2.28 mg. on December 9, or 24 per cent. The rise in Experiment III was inconsiderable, 8.7 per cent.

Figure 2 shows the changes in plasma amino N that occurred when the protein or the protein and energy content of the rations were again increased. In Experiment III, in which the quantity of the dietary protein was increased 91 per cent without change in its quality, there was no change in plasma amino N. In Experiments V and VI, in which the quality of the dietary protein was improved along with a similar increase in its quantity (102 per cent in the former experiment and 92 per cent in the latter), the plasma amino N actually dropped decidedly. In Experiment V it dropped from 2.53 mg. to 1.81 mg., or 28 per cent, and in Experiment VI from 2.28 mg. to 1.9 mg., or 17 per cent. From these data the following conclusions may be drawn:

1. *When a milking cow has been on an inadequate protein ration for some time, the quantity of the dietary protein may be increased 90 to 100 per cent without causing an increase in plasma amino N. The latter may even drop decidedly.*

2. *The quality of the dietary proteins under certain conditions affects decidedly the plasma amino N, and the ration containing the more efficient protein mixture is associated with the lower level of plasma amino N.*

3. *The level of amino N associated with a given ration depends largely upon the nutritional condition of the animal as affected by dietary history.* This is shown best by comparing periods 1 and 3 of Experiments I and V (Tables III and VII). In period 1 of Experiment V the plasma amino N was 2.49 mg. In the first determination of period 3 on the same ration it was 1.81 mg. The corresponding data for Experiment I are 2.3 mg. and 1.57 mg., respectively.

When the quantities of the protein in the rations in Experiments V and VI were increased, at least two factors probably contributed to the drop in plasma amino N that occurred: (1) The improvement in the quality of the dietary protein, and (2) the nutritional condition of the animal which was produced by the preceding period of reduced feeding.

In Experiment I, in which the energy content of the ration was increased simultaneously with the quantity of protein, the plasma amino N apparently at first did not change. At the end of period 2 it was 1.66 mg. Three days after the change of ration it was 1.57 mg. Later it rose to 2.64 mg. without further change of ration. The increase in the energy content of the ration in this experiment much more

TABLE VI.—*Experiment IV, cow 246. Quantity of protein in ration reduced. No change in quality*

Period No.	Date	Milk (daily)					Amino N[a]		Tryptophane			
									Blood		Plasma	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	Individual determinations (mg. per 100 c. c.)	Average (mg. per 100 c. c.)	Individual determinations (mg. per 100 c. c.)	Average (mg. per 100 c. c.)
	1922	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*						
1	Oct. 27–31	10.16										
1	Nov. 1	10.34							1.03,1.04,1.00	1.02		
1	Nov. 2, 3	10.10										
1	Nov. 4, a. m.	9.84										
2	Nov. 4, p. m.											
2	Nov. 5	9.43										
2	Nov. 6	8.98										
2	Nov. 7	8.57										
2	Nov. 8	8.62										
2	Nov. 9	8.30										
2	Nov. 10	8.57										
2	Nov. 11	8.21	4.31	355	0.546	44.9					1.00,1.08	1.04
2	Nov. 13	8.48	3.88	329	.514	43.6		1.84				
2	Nov. 14	8.62	3.87	334	.528	45.5	4.58	1.95	1.456, 1.464	1.46		
2	Nov. 15–19	8.97	3.86	346	.548	49.2						
2	Nov. 20	8.39	3.89	326	.555	46.6		2.20	1.03, 1.05	1.04		
2	Nov. 23–26	7.78	3.92	305	.553	43.0						
2	Nov. 27	7.26	4.04	293	.550	39.9	4.67	2.31				
2	Nov. 30–Dec. 4	7.54	3.86	291	.552	41.5						
2	Dec. 6	7.62	3.62	276	.543	41.3	[b] 2.67	2.89	0.92, 0.73	.82		
2	Dec. 7–8	7.94	4.02	319	.544	43.2						
2	Dec. 9	7.76	3.91	303	.544	42.2	3.93	2.28	0.64, 0.62	.63		

[a] Average difference in duplicate amino-N determinations in blood 7.9 per cent, and in plasma 6.1 per cent.
[b] This unusual blood amino-N is substantiated by four determinations made by two different methods. It is difficult to see how it could be due to accident in analytical work.

TABLE VII.—*Experiment V, cow 54. Change in the quantity and quality of protein in ration*

Period No.	Date	Milk (daily)							Amino N[a]		Sugar	
		Yield	Nitrogen		Fat		Lactose		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1920		*P. ct.*	*Gm.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*				
1	Oct 22–25	12.84										
1	Oct. 26	13.25	0.4965	65.76	3.49	462.3			4.01	2.43		
1	Oct. 27	13.06	.4864	63.54	3.58	467.7						
1	Oct 28	12.97	.4988	64.72	3.48	461.5			4.34	2.55		
2	Oct 29	12.47	.4843	60.42	3.46	431.6	5.28	658.6				
2	Oct. 30	12.57	.4659	58.54	3.46	434.8						
2	Oct. 31	12.25	.4499	55.10	3.34	409.1	5.28	646.6				
2	Nov. 1	11.79	.4600	54.25	3.22	379.8						
2	Nov. 2	10.93	.4545	49.68	3.21	350.9			4.06	2.47		
2	Nov. 3	12.07	.4478	54.04	2.94	354.7	5.23	631.1				
2	Nov. 4	10.52	.4346	45.74	3.12	328.4						
2	Nov. 5	10.80	.4059	43.82	3.06	330.4						
2	Nov. 7	10.89	.4699	51.15	3.43	373.9	5.36	583.5				
2	Nov. 8	11.52										
2	Nov. 9	11.78	.4467	52.68	2.91	342.9						
2	Nov. 11	12.11	.4409	53.40								
2	Nov. 12	11.20	.4499	50.40	2.89	323.8	5.39	603.9				
2	Nov. 13	11.30							4.42	2.53		
3	Nov. 14	11.30										
3	Nov. 16	11.34	.4767	54.06	3.01	341.3	5.39	611.2				
3	Nov. 17	12.16	.4565	55.49	3.08	374.4						
3	Nov. 18	12.25	.4709	57.67	3.06	374.8						
3	Nov. 19	12.66	.4748	60.09	3.11	393.6						
3	Nov. 21 22	12.45										
3	Nov. 23	12.61	.4672	58.92	3.20	403.5	5.23	659.5				
3	Nov. 24	12.02	.4489	53.96	3.39	407.5			3.63	1.81	64.0	75.5
3	Nov. 25	11.66										
3	Nov. 26	11.34	.4837	54.85	3.44	390.1	5.23	593.1				
3	Nov. 27	11.84	.4946	58.55	3.64	430.9	5.34	632.2	4.05	1.95	64.1	77.1

[a] Average difference in duplicate amino N determinations in blood 3.4 per cent, and in plasma 9.3 per cent.

than offset the increased output of energy due to the rise in milk yield which took place in the third period. The data here, therefore, exclude the increased energy requirement for the increased milk yield in Experiments V and VI as a considerable factor tending to effect the drop in plasma amino N produced by increasing the dietary protein.

From the data in Figures 1 and 2 it appears that with milking cows, where absorption is a fairly constant and continuous process, and where there is a constant demand for energy for milk secretion, the plasma amino N is more affected by (1) the energy content of the ration, (2) the quality of the dietary protein, and (3) the nutritional condition of the animal than it is by the actual amount of protein in the ration. Increases in the quantity of dietary protein are not infrequently followed by decreases in the concentration of the plasma amino N, and it seems a rather general rule that a lower level of plasma amino N is associated with a higher efficiency of the dietary protein for milk secretion.

It must be remembered that all of these experiments were made on milking animals, and it may be pointed out that a low plasma amino N associated with a high efficiency of dietary protein is what one would expect in such animals on the supposition that the efficiency for milk secretion of the plasma mixture of amino acids varies in the same direction as the efficiency of the dietary protein. When the efficiency for milk secretion of the plasma amino acid mixture was high, the mammary gland would take out a large proportion of the total amino N for the synthesis of the milk proteins, and less would be left in the circulation. The results suggest, therefore, that the quality as well as the quantity of the plasma amino acid mixture is subject to variation.

TABLE VIII.—*Experiment VI, cow 246. Increase in quantity and quality of protein in ration*

Period No.	Date	Milk (daily)					Amino N [a]		Tryptophane	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c.c.)	Plasma (mg. per 100 c.c.)	Individual determinations (mg. per 100 c.c.)	Average (mg. per 100 c.c.)
	1922	*Kg.*	*P. ct.*	*Gm.*	*P. ct.*	*Gm.*				
1	Dec. 2–8	7.72	3.84	297	0.546	42.1				
1	Dec. 9	7.76	3.91	303	.544	42.2	3.93	2.28	0.64, 0.62	0.63
2	Dec. 10									
2	Dec. 11	7.98	3.76	300	.554	44.2				
2	Dec. 13	8.48	3.88	329	.551	46.8	3.93	1.90	.69, .59	.64
2	Dec. 14	8.53	3.91	333	.549	46.8				
2	Dec. 15–19	7.64	4.02	298	.569	42.2				
2	Dec. 20	7.44	3.96	295	.588	43.8	4.01	2.10	1.01, .96, 1.03	1.00
2	Dec. 22	7.94	3.97	315	.564	44.8				
3	Dec. 23–28	7.99	3.90	318	.558	45.5				
3	Dec. 29 [b]									
	1923									
3	Jan. 7	7.51	3.92	302	.577	43.3				
3	Jan 8	6.53	3.88	271	.604	39.5	4.06	2.03	.65, .62	.63
3	Jan. 9–18	6.73	4.03	271	.607	40.9				
3	Jan. 27–Feb. 5	6.62	4.08	269	.600	39.9				
3	Feb. 6	6.80	4.00	272	.596	40.6	4.14	2.26	.83, .80, .74	.79
3	Feb. 9–15	6.93	3.93	268	.581	40.3				
3	Feb. 16	7.35	4.17	306	.614	45.1	3.63	1.98	.95, .86	.90
3	Feb. 18–21	6.89								
4	Feb. 22–28	6.81								
4	Mar. 1–6	7.32								
4	Mar. 7–12	7.05								
4	Mar. 13	7.39					3.83	2.22	1.201, 1.205	1.20

[a] Average difference in duplicate amino-N determinations in blood 4.5 per cent, and in plasma 3.3 per cent.

[b] Off feed. Refused 1.66 kg. of grain on Dec. 30; 2.29 kg. of grain on Dec. 31; and 2.37 kg. of grain, 1.18 kg. of hay, and 5.91 kg. of silage on Jan. 1.

In order to test this hypothesis, the free tryptophane and the total amino N of the blood have been followed simultaneously in Experiments IV, VI, and VIII. (See Tables VI, VIII, and X.) From the results of these experiments the following conclusions may be drawn:

1. *Under appropriate conditions very great changes may occur in the concentration of the free tryptophane in the blood while at the same time relatively little or no change occurs in the blood amino N.* In Experiment IV the tryptophane in the blood on November 14 was 1.46 mg. per 100 c. c., and on December 9 it was 0.63 mg., a difference of 57 per cent. The difference in blood amino N between these dates was only 14 per cent. In Experiment VI the blood tryptophane increased from 0.63 to 1.2 mg., or 90 per cent, with no increase in blood amino N; and in Experiment VIII the blood tryptophane increased about 100 per cent between periods 1 and 3 with practically no change in blood amino N.

2. *These alterations in the composition of the blood mixture of amino acids may, under certain conditions, continue for weeks.* On the low-protein ration in period 3 of Experiment IV the blood tryptophane dropped from 1.46 mg. to 1.04, 0.82, and 0.63 mg. in four consecutive samples taken during a period of 25 days. This drop in blood tryptophane was probably caused by a decrease in the rate at which the tissues yielded tryptophane to the blood.

3. *These experiments show that changes occur in the composition of the blood mixture of amino acids (a) when the quantity of protein in the ration is reduced, (b) when the quality of the dietary protein is changed, and (c) when the quantity of protein in the ration is increased.*

The rise in blood tryptophane between periods 1 and 2 from 1.03 to 1.46 mg. in Experiment IV, and from 0.34 to 0.45 mg. in Experiment VIII, might well be expected even in view of the fact that the quantities of tryptophane in the protein of the rations were low in the first periods of both experiments and still lower in the second periods. An amino acid like valine, which is lower relatively in both muscle and corn proteins than in milk proteins and is absent from gelatin, might well determine the extent of increase in negative N balance in changing from the rations of the first period to those of the second period, and thus might effect a rise in blood tryptophane. This explanation accords with that given above for the subsequent drop in blood tryptophane in period 2 of Experiment IV.

TABLE IX.—*Experiment VII, cow 246. Change in quality of protein in ration; no change in quantity*

Period No.	Date	Milk (daily)					Amino N [a]	
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)
	1922	*Kg.*	*Per cent*	*Grams*	*Per cent*	*Grams*		
1	September 4–8	12.59	3.41	420	0.440	54.5		
1	September 9	12.70	3.67	466	.440	56.0	3.98	
1	September 10–12	11.90	3.78	454	.447	53.6		
1	September 13 [b]	11.79	3.71	436	.456	53.8	3.66	1.83
2	September 14	12.11	3.86	468	.453	54.9		
2	September 15	11.93	3.63	433	.438	52.3		
2	September 16	11.61	3.40	395	.438	50.9		
2	September 17	11.66						
2	September 18	11.52	3.98	459	.462	53.2		
2	September 19	11.52	4.04	465	.456	52.5	4.37	2.43
2	September 20	11.52	3.83	441	.451	52.0		
2	September 21	11.48	3.86	443	.435	49.9		
2	September 22	11.39	3.82	435	.453	51.6		
2	September 23	11.30	4.28	483	.459	51.8		
2	September 24	11.48	4.06	466	.471	54.0		
2	September 25	11.25	3.93	442	.473	53.2		2.08
2	September 26	11.20	4.17	467	.497	55.6		
2	September 27	11.34						
2	September 28	10.89	3.78	412	.502	54.6		

[a] Average difference in duplicate amino-N determinations in blood 2.9 per cent, and in plasma 2.9 per cent.

[b] On Sept. 13, 0.45 kg. of grain was refused

As a matter of convenience, the qualitative changes in the mixture of amino acids in the whole blood rather than in the blood plasma have been studied. The conclusions, that have been reached, however, are undoubtedly applicable to the blood plasma as well.

the ration was changed; but in Experiment VIII, where the energy of the ration and the plasma amino N were higher, the total amino N did not rise. In fact, there was no significant change in plasma amino N throughout the latter experiment until after the corn-milk protein ration of period 3 had

TABLE X.—*Experiment VIII, cow 423. Change in quality of protein in ration; no change in quantity*

Period No.	Date	Milk (daily)					Amino N [a]		Tryptophane (mg. per 100 c. c.)
		Yield	Fat		Nitrogen		Blood (mg. per 100 c. c.)	Plasma (mg. per 100 c. c.)	
	1921	*Kg.*	*Per cent*	*Gm.*	*Per cent*	*Gm.*			
1	Nov. 5, 6	7.01	5.51	386	0.626	43.8			
1	Nov. 8	7.21	5.57	402	.632	45.6	4.37	2.46	0.34
1	Nov. 10–11	6.94	5.77	400	.625	41.0			
1	Nov. 12	6.89	5.73	395	.651	44.9	4.20	2.24	
2	Nov. 13	6.89	5.75	396	.647	44.6			
2	Nov. 14	6.35	5.62	357	.679	43.2			
2	Nov. 15	6.26	4.98	312	.672	42.1			
2	Nov. 17	5.72	6.59	376	.738	42.2			
2	Nov. 18	5.40	6.55	354	.719	38.8			
2	Nov. 19	5.26	6.19	326					
2	Nov. 20	5.76	7.44	429					
2	Nov. 21	5.44	6.21	338	.692	37.7			
2	Nov. 22	5.44	6.04	328	.672	36.5	4.22	2.37	.45
2	Nov. 23	5.22	6.42	335	.688	35.9			
3	Nov. 24	5.49	6.22	341	.658	36.1			
3	Nov. 25	5.90	5.96	351	.623	36.7			
3	Nov. 26	6.21	5.43	338					
3	Nov. 27	6.49	5.07	329	.637	41.3			
3	Nov. 28	6.49	5.69	369	.631	40.9			
3	Nov. 29	6.26	5.02	314					
3	Nov. 30	6.26	5.02	314					
3	Dec. 1	6.44	5.07	326	.638	41.1	4.14	2.28	.66
3	Dec. 3	6.53	5.35	350					
3	Dec. 4	5.94	5.35	318	.626	37.2			
3	Dec. 5	5.44	5.54	302	.653	35.6	4.57	3.08	.69

[a] Average difference in duplicate amino-N determinations in blood 2.9 per cent, and in plasma 1.6 per cent.

Although it was found, as was to have been expected, that a high plasma amino N is, in general, associated with a dietary protein mixture of low efficiency, there are a number of circumstances in which this might not be true. Thus in Experiments VII and VIII (Tables IX and X) in changing from the first to the second period the quality of the dietary protein was reduced with no change in quantity; the milk yield was reduced; the concentrations of certain amino acids in the blood plasma were reduced, whereas the concentrations of others were increased; and, whether the total plasma amino N would increase or not, one can readily see, would largely depend upon the responsiveness of the mechanism destroying these latter amino acids to the increases in their concentrations. In Experiment VII, where the energy of the ration and the plasma amino N were low, the total amino N rose when

been fed for a considerable time. The abrupt rise from 2.28 to 3.08 mg. per 100 c. c. at this time probably marks a change in the storage capacity of the tissues.

CHANGES IN THE YIELD AND COMPOSITION OF MILK

Figure 3 shows that in Experiments I to V, when the protein or energy, or both, in the rations were reduced, the milk yields dropped decidedly; figure 4 shows that this effect on the milk yields was reversed when these dietary changes were reversed; and Figure 5 shows that the milk yields varied with the quality of the dietary protein in Experiments VII and VIII, in which the quality of the dietary protein was changed without change in its quantity or in the energy of the ration.

Figures 6 to 13 show the changes in the composition of the milk in all ex-

cept Experiment IV, where no determinations were made in period 1. The results may be summarized as follows:

1. Experiments II and V show that the concentration of lactose in the milk of an individual cow is exceedingly constant and is unaffected by wide variations either in the quantity and quality of the dietary protein or in the quantity of carbohydrate in the ration. It varied in these two experiments from 5.23 per cent to 5.42 per cent, or 3.6 per cent difference.

2. A reduction in the energy content of the ration, either alone (Experiment II) or together with the protein (Experiment I), has no constant tendency to reduce the concentration of milk fat, but does effect a reduction in the concentration of milk N. In Experiment I the concentration of milk fat was higher on the ration low in its protein and energy content than on the original ration.

3. A reduction in the quantity (Experiment III) or quantity and quality (Experiment V) of the dietary protein, without altering the energy content of the ration, reduces the concentrations of milk N and fat.

4. In Experiments VII and VIII, where only the quality of the dietary protein was changed, the concentrations of milk N and fat were higher when the dietary protein was qualitatively less well adapted to the secretion of the milk proteins.

5. In all these experiments where only the protein of the ration was altered there is in general a parallelism between the changes in the concentration of milk N and fat that is certainly quite evident.

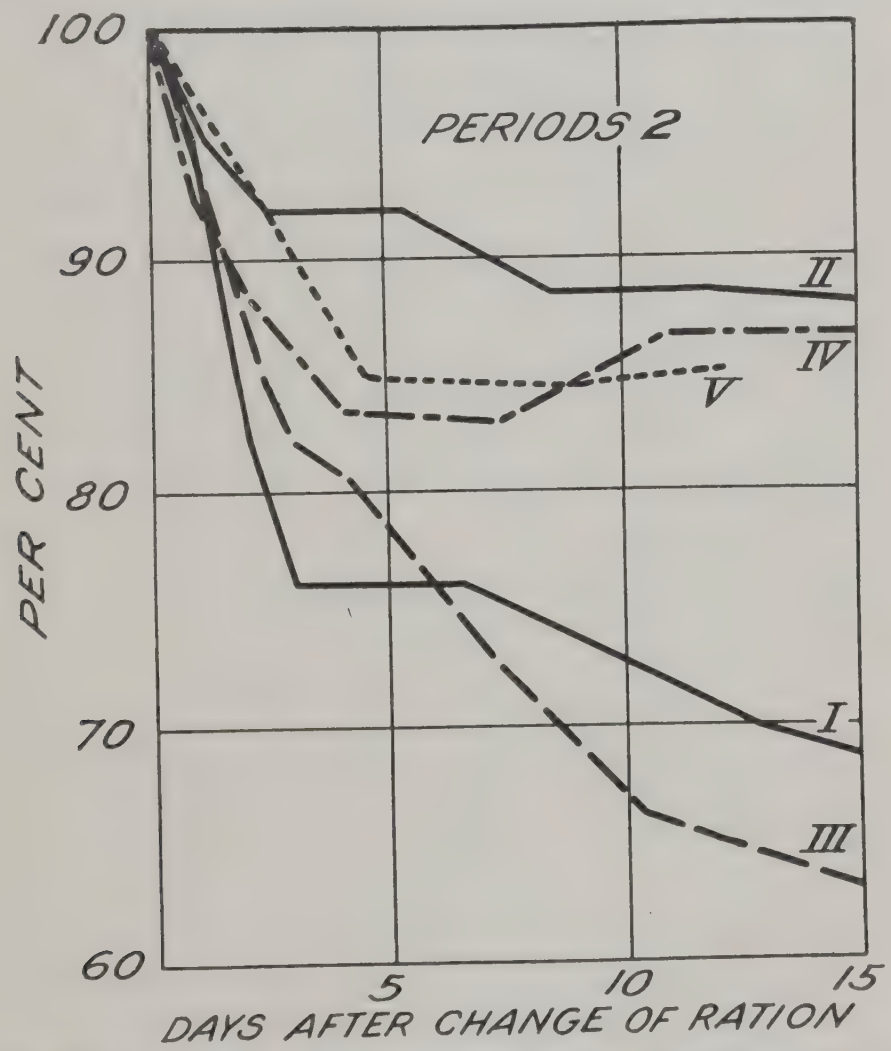

FIG. 3.—Experiments I–V. Change of milk yield when amount of protein or energy, or both, were reduced in the ration after period on adequate ration. The yield before the ration was changed is taken as 100 per cent

RELATION BETWEEN THE COMPOSITION OF BLOOD AND THE SECRETION OF MILK

The results of these experiments show, as was to have been expected, that the relations between the diet, the composition of the blood, and the secretion of milk are exceedingly complicated. We are still very far

from knowing all the factors which affect these relationships, and the physiological conclusions to be drawn from such experiments as those which have just been presented must, therefore, be more or less tentative for a long time to come. But the manner in which diet affects the composition of the blood and in which this again affects the various bodily activities are matters of such fundamental physiological importance that it will be profitable to determine and record the facts in this field even though their full significance cannot be realized until they are supplemented by additional work more extensive than can be carried out by a single investigator. In the meantime it is worth while to set forth some of the conclusions which appear to be reasonably justified, though perhaps not fully established, by the work done so far.

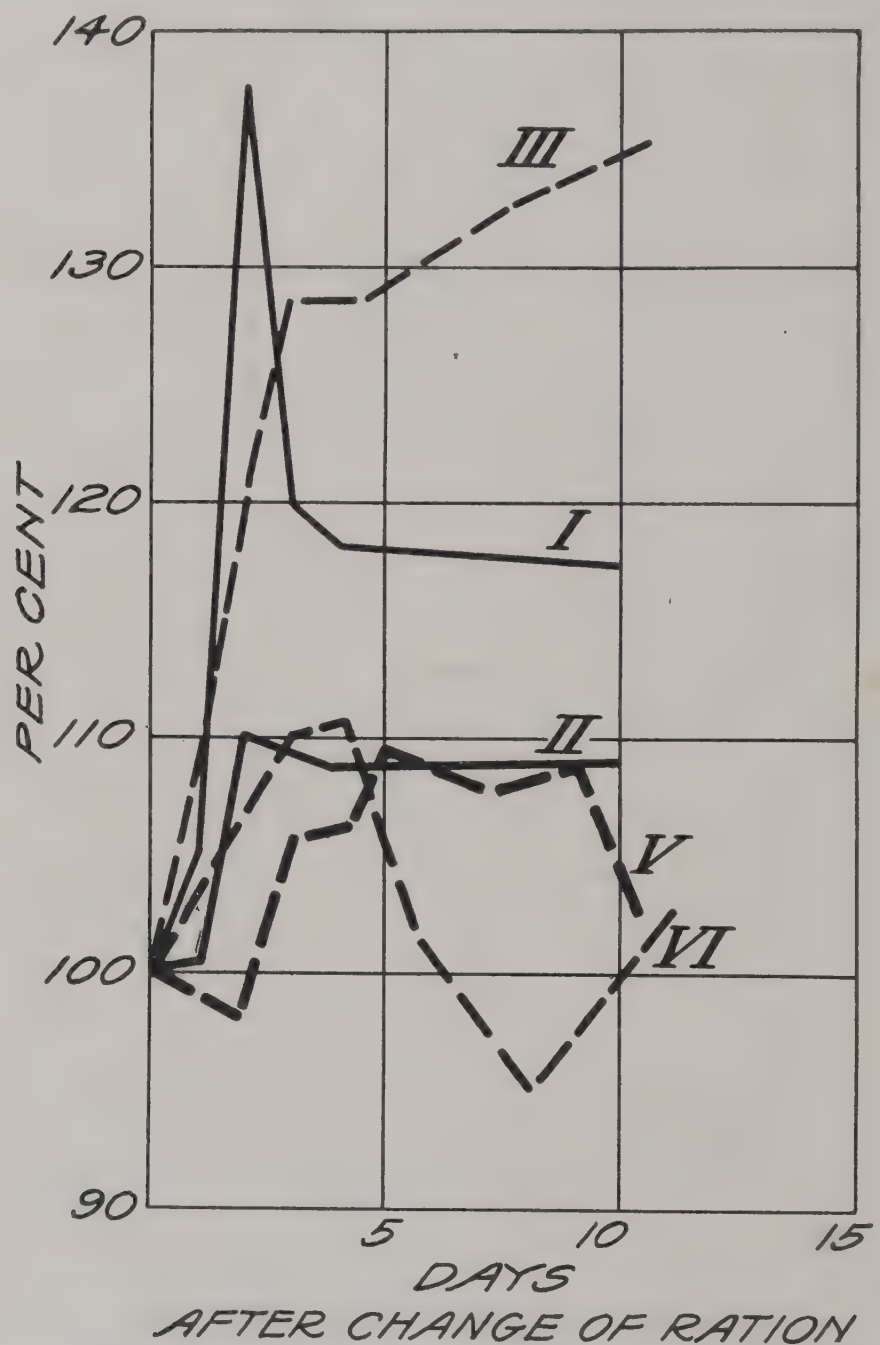

FIG. 4.—Experiments I, II, III, V, and VI. Change of milk yield when amount of protein or energy, or both, were increased in ration after a period of reduced feeding. Yields at end of previous period taken as 100 per cent

In order to interpret the results which are to be considered in this section, it is necessary to bear in mind that the lactose, fat, and proteins of milk are made in the mammary gland from glucose, phosphatide, and amino acids, respectively, that are taken from the plasma of the blood.

It is necessary also to bear in mind certain evidence, which will be reviewed briefly at this point, that indicates that the secretion of milk may be affected through either the precursor of milk fat or that of milk protein. This evidence gives some idea of the differences in the changes that occur in the composition of milk when its secretion is affected by these different precursors.

Morgen and a number of his collaborators have conducted an extensive series of experiments on sheep and goats in which the effects on milk yield of varying the protein and fat of the diet were studied (*13–16*). The plan of the experiments was to change the rations by substituting protein or fat for an isodynamic amount of carbohydrate, or vice versa, and then, after an interval of some days, to determine the effect which had been brought about on the yield and composition of milk. It was found that milk yield could be affected by such changes in either the protein or fat of the ration. Changes in the dietary protein affected the milk yield through a wide range in the level of the protein feeding; changes in the dietary fat, on the other hand, only when they were made at low levels of fat feeding. Changes in the dietary protein produced only irregular changes in the composition of the milk; changes in the dietary fat, when they produced changes in milk yield at all, generally changed the concentration of milk fat in the same direction as the change made in the dietary fat; and the concentration of milk nitrogen, in the opposite direction. It seems likely that the changes in the composition of milk produced by changes in the quantity of dietary fat would have been even more regular than they were had they been determined immediately instead of after an interval of some days. The concentration of lactose in the milk was not significantly affected by any of the dietary changes studied in Morgen's experiments.

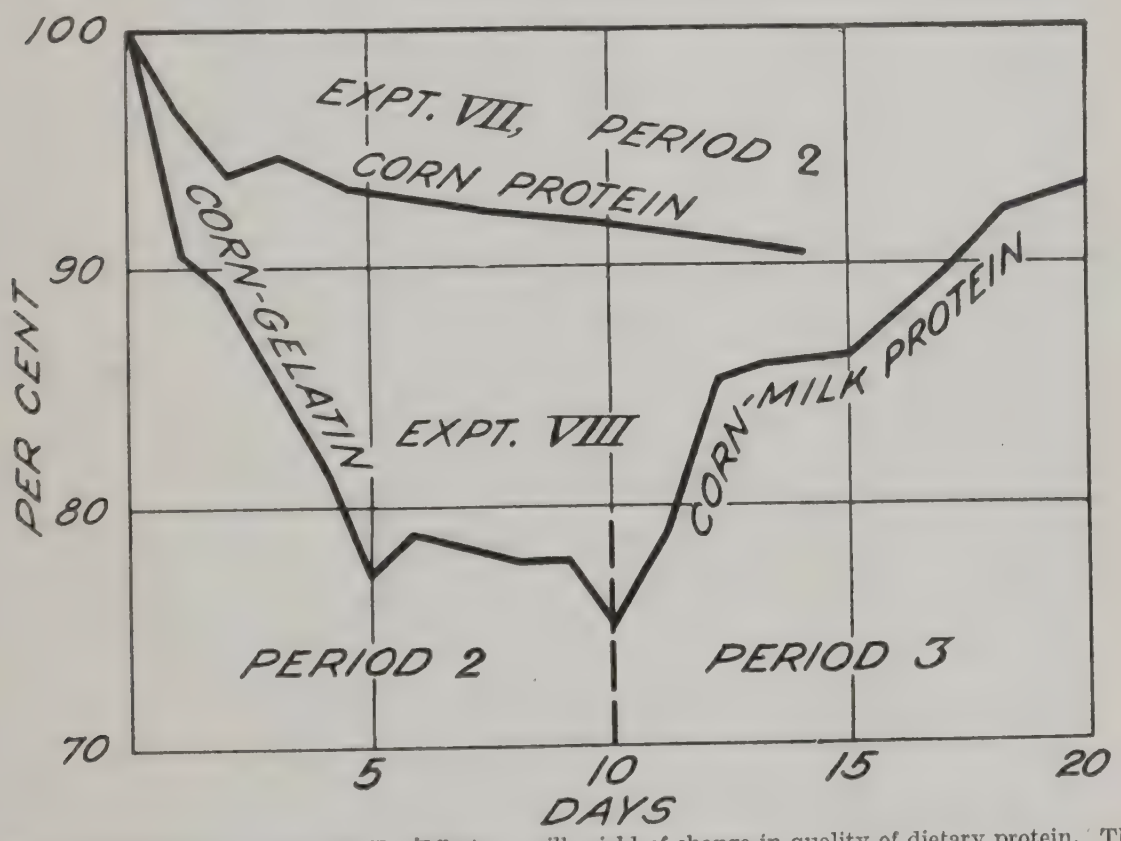

FIG. 5.—Experiments VII and VIII. Effect on milk yield of change in quality of dietary protein. The yield before the ration was changed is taken as 100 per cent

Subsequent to Morgen's work, an extensive series of experiments, in which the effects of dietary changes in fat on the yield and composition of cow's milk were studied, was made by the Deutscher Landwirtschaftsrat (*4*). The results of these experiments indicate that the dietary fat has less effect on milk secretion in cows than it has in sheep and goats. But an experiment reported by Jordan and Jenter (*9*) indicates that, under appropriate circumstances, milk secretion in cows may be affected by changes in dietary fat and that, when it is so affected, the concentration of milk nitrogen varies in a direction opposite to that of the change made in the dietary fat.

Experiments reported in the present article and discussed to some extent in the preceding pages show that milk secretion in cows is markedly affected by changes in the dietary protein.

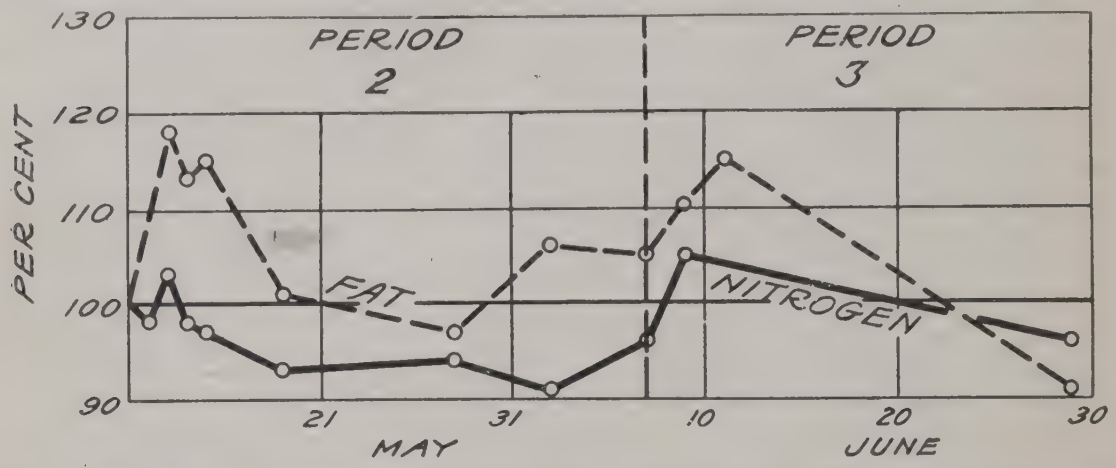

FIG. 6.—Experiment I. Changes in concentration of nitrogen and fat of milk. The concentration in period 1 is taken as 100 per cent. In period 2 the protein and energy of the ration were reduced, and in period 3 they were restored to adequacy

In general, it may be said that milk secretion may be affected by changes in either the fat or protein of the ration, but much more easily by changes in the protein. When the milk yield is affected by changes in the dietary fat, there is generally a tendency for the concentration of milk nitrogen to vary in a direction opposite to that of the change made in the dietary fat, while this is not the case when milk yield is affected by changes in the dietary protein. The situation is most easily accounted for by supposing that a change in the protein of the ration tends to affect milk secretion through a change in the precursor of milk protein—namely, the free amino acids of the blood—while a change in the dietary fat tends to affect it through a change in the precursor of milk fat—namely, the phosphatide of the blood. These considerations justify the attempt to interpret the results which are to be discussed in this section on the hypothesis that changes in diet frequently affect milk yield through changes brought about in the free amino acids of the blood.

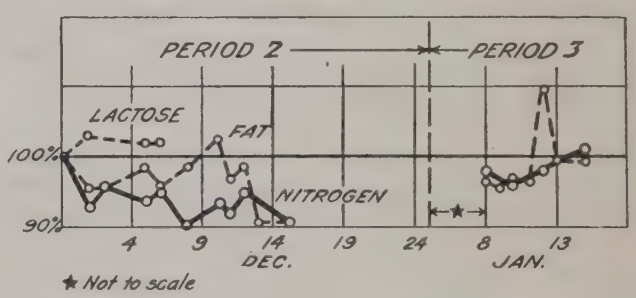

FIG. 7.—Experiment II. Changes in the composition of milk. The concentration in period 1 is taken as 100 per cent. In period 2, the energy content of the ration was reduced, while the protein was left unchanged; in period 3, the energy was restored to adequacy

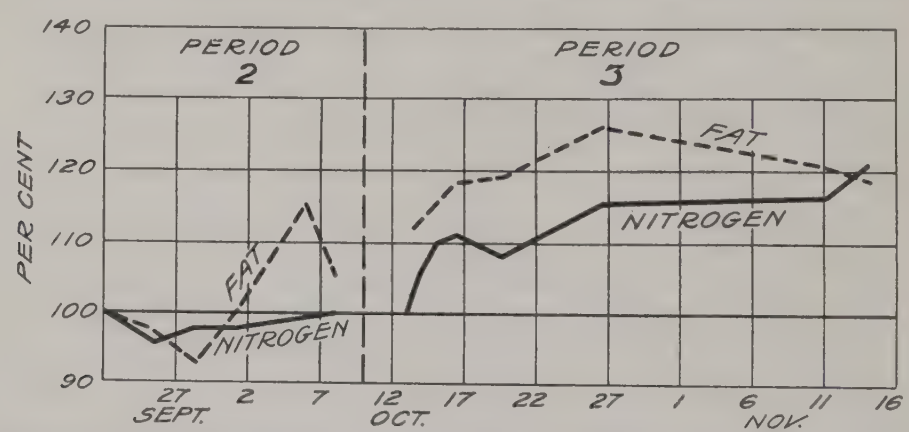

FIG. 8.—Experiment III. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2, the quantity of protein in the ration was reduced without change in its quality; in period 3, it was restored to adequacy

The discussion of the individual experiments of this series may well be begun with that of Experiment II, in which the quantity of energy (chiefly carbohydrate) in the ration was markedly reduced without making any con-

siderable change in the protein. As a result there was a drop in the concentration of amino N in the blood and plasma, a drop in milk yield, and a drop in the concentration of milk N (figs. 1, 2, and 7). When the energy of the ration was restored to the initial level, the amino N of the blood and plasma, the milk yield, and the concentration of milk N all rose (figs. 4 and 7). The amino N in the blood and plasma was determined so long after the second change in ration that its rise has not been shown in any of the figures, but it may be seen in Table IV. The results of this experiment may be taken as particularly strong and consistent evidence for the view that the free amino acids of the blood play a predominant part in regulating milk secretion. This is rather surprising when we bear in mind that only the energy content of the ration in this experiment was changed.

In Experiments III and IV the quantity of dietary protein was reduced without change in its quality and without change in the dietary energy; and in Experiment III the protein was after a time restored to the initial level. In Experiment I the dietary protein and energy were reduced together and later restored to the initial level. In all three of these experiments the milk yield and the concentration of milk N followed the changes in dietary protein in the same sense in which they followed the dietary energy in Experiment II (figs. 3, 4, 6, and 8). In all three cases, also, there is reason to think that the reduction in dietary protein was followed by a reduction in the level of the plasma amino N (fig. 1). These results are therefore like those of Experiment II, consistent with the view that the dietary changes affected milk yield through the free amino acids of the blood.

In all three of these experiments, however, the plasma amino N began to rise while the cows were still on the inadequate rations and while the milk yields in two of the cases were still falling off (figs. 1 and 3). Further, when the rations were restored to the initial levels in Experiments I and III, there was a marked tendency for the milk yields and the concentration of milk N to rise in both cases, though in neither case was there any tendency for the plasma amino N to rise.

The results of Experiments V and VI how even more clearly that there is no hard and fast relation between the rate of milk secretion and the concentration of total amino N in the blood plasma. In these experiments both the quantity and the quality of the dietary protein were changed. When the quantity and quality of the dietary protein were reduced together, there was a decided tendency for the milk yield to fall off and for the concentration of milk N to be reduced; and both these tendencies were reversed when the quantity and quality of the dietary protein were increased (figs. 3, 4, 9, and 10). But reduction in the quantity and quality of the dietary protein caused no significant change in the level of plasma amino N (fig. 1); and the opposite changes in the dietary protein were followed in both experiments by decided reductions in the level of plasma amino N (fig. 2) in spite of the rising milk yields and rising concentrations of milk N.

The lack of parallelism between plasma amino N and milk yield, which is to some extent apparent in all the

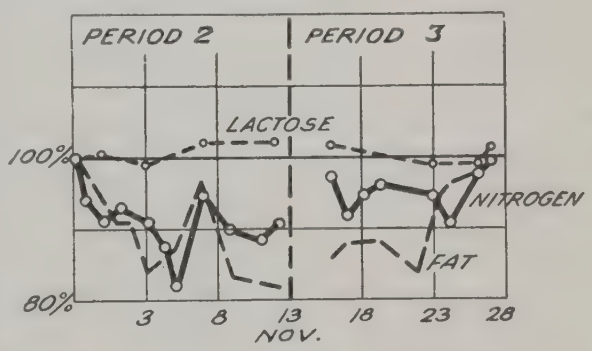

FIG. 9.—Experiment V. Changes in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the protein of the ration was reduced in both quantity and quality; in period 3 it was restored to adequacy

experiments, but particularly in experiments V and VI, is no real reason for abandoning the hypothesis that in these experiments the milk yield was controlled through the plasma amino-acid mixture. The manner in which milk yield may be increased by a change in the plasma amino-acid mixture, even though the concentration of total plasma amino N may be at the same time reduced, is shown by the results of Experiments IV, VI, and VIII, in which the plasma amino N and the free tryptophane of the blood were followed simultaneously. (Tables VI, VIII, and X.) These experiments have already been discussed to some extent. They show that when the quantity of protein in the ration is reduced, the composition of the blood mixture of amino acids may be very considerably altered; that when the milk yield falls off rapidly during a period on a ration inadequate in protein, and the plasma amino N at the same time gradually rises, the concentration of tryptophane in the blood may constantly fall; that when

the dietary protein is increased and the milk yield increases without a rise in total plasma amino N, the concentration of blood tryptophane may rise; and, finally, that when the milk yield is altered by a change in the quality of the dietary protein, the concentration of blood tryptophane may be very greatly altered, although the total plasma amino N may be unchanged. The correspondence between the changes of diet on the one hand and of the yield and composition of milk on the other, taken along with these results which show that the quality of the plasma amino acid mixture may vary as well as its quantity, justify the belief that the changes in dietary protein in these experiments influenced the yield of milk largely through changes in the quality of the plasma amino-acid mixture.

In Experiments VII and VIII the quality of the dietary protein was changed without change in its quantity. The resulting changes in plasma amino N and in total milk yield are in fairly close agreement with the conclusions which have been drawn from the other experiments, and no special comment is required. But the concentration of milk N decreased in these two experiments when the quality of the dietary protein was improved, and vice versa, and this is not in entire accord with the results of the other experiments. The factors which control the concentration of milk N are, however, in all probability numerous and complicated, and it is not felt that the lack of parallelism between quality of dietary protein and concentration of milk N in these two experiments is sufficient seriously to impair the force of the conclusions which have been drawn from the others.

This part of the discussion may be concluded by pointing out certain other interesting aspects of the results. When changes in milk secretion were brought about by altering the dietary protein without changing the total energy of the ration, there was a strong tendency for the concentrations of milk N and fat to run parallel (figs. 8 to 12). There is no reason to think that changes in dietary fat had any influence on the concentration of milk fat in these experiments, for, although small changes were frequently made in the dietary fat at the same time that the protein was altered, there was no tendency toward parallelism between changes in dietary fat and changes in milk fat. In addition, the experiments of the Deutscher Landwirtschaftsrat (*4*) referred to above show that the changes which were made in the dietary fat in these experiments were not of such a nature that they could have had any influence either on the secretion of milk fat or on milk secretion in general. They also indicate that it was not through changes in the amount of fat formed from protein or through the phosphatide of the blood plasma that the changes in dietary protein affected the secretion of milk fat. The results, therefore, indicate that the secretion of milk fat may be influenced through the amino-acid mixture of the blood. It must be pointed out, however, that in Experiment I, where the protein and energy of the ration were changed together, and in Experiment II, where the energy was changed while the protein was kept constant, there was no marked tendency toward parallelism between the concentrations of milk protein and milk fat. Considerable changes in the concentration of plasma amino N had here no tendency to affect the secretion of milk fat. It seems not unreasonable to suppose that reductions in the energy of a ration tend to cause body fat to be thrown out into the blood and that this increased supply of fat in the blood may overcome the tendency for the concentration of milk fat to go down with that of the milk nitrogen. This is suggested by the work of Eckles and

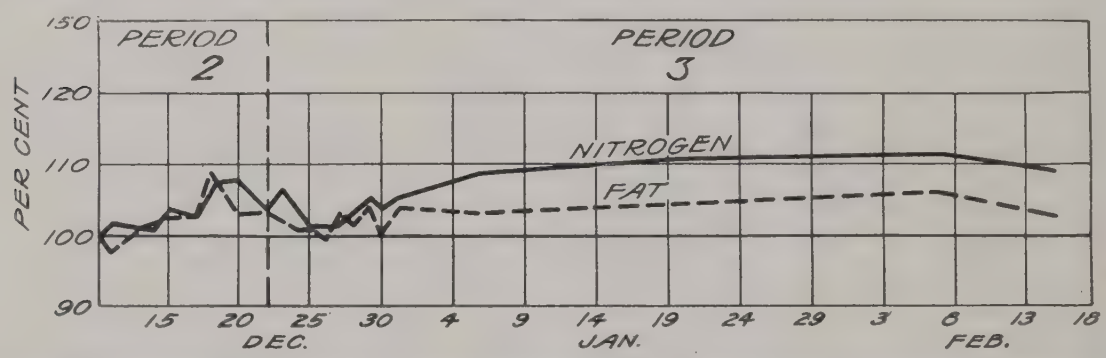

FIG. 10.—Experiment VI. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the quantity of protein in the ration was increased and its quality improved; in period 3, the quantity and quality of the ration were still further increased

Palmer (*5*), in which they found that when the protein and energy of the ration of milking cows were reduced as in Experiment I the composition of the milk fat approached that of body fat, and further, by the relatively small drop in plasma phosphatide as compared with that of the plasma amino-N in Experiment I.

Another interesting indication which is given by the results of Experiments I, II, and III as shown in Figures 1 and 3 is that reductions in dietary protein tend chiefly to reduce the efficiency for milk secretion of the plasma amino-acid mixture, while reductions in dietary energy tend to reduce the quantity of plasma amino N and perhaps, at the the same time, to increase the inefficiency for milk secretion of the plasma amino-acid mixture. In Experiment I the dietary protein and energy were reduced together, there was a very large reduction in plasma amino N, and a large reduction in milk secretion. In Experiment III the dietary protein alone was reduced, there was a small reduction in plasma amino N, but a reduction in milk secretion even a little larger than in Experiment I. In Experiment II the dietary energy alone was reduced, the plasma amino N fell far below the normal level, while the reduction in milk secretion was quite small. It seems difficult to explain these relations, except on the supposition that the efficiency for milk secretion of the plasma amino-acid mixture was much higher in Experiments I and II, where the dietary energy was reduced, than in Experiment III, where the protein was reduced while the energy was kept constant.

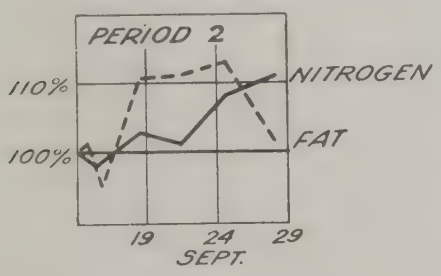

FIG. 11.—Experiment VII. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the quality of the protein fed was not so good as in period 1; its quantity was unchanged

SUMMARY

In experiments on milking cows, changes have been made in the energy and protein of the rations both separately and simultaneously. The protein has been changed in quantity or quality, or both. The effects of these changes on the composition and yield of milk, on the concentration of amino N of the blood and plasma, and, in some cases, on the concentration of free tryptophane and other constituents in the blood, have been determined. The results obtained may be summarized as follows:

1. A sharp cut in the energy content of the ration of a milking cow produces a decided reduction in the plasma amino N; with milking cows the energy content of the ration is an important factor in determining the level of plasma amino N.

2. A sharp cut in the quantity of protein in the ration produced only a slight drop in plasma amino N when the quality of the protein was unchanged, and no drop at all when the quality of the dietary protein was reduced along with the quantity.

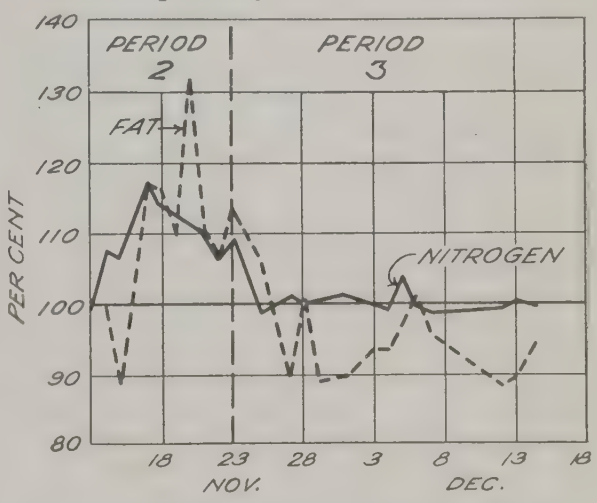

FIG. 12.—Experiment VIII. Change in the composition of milk. Concentration in period 1 is taken as 100 per cent. In period 2 the quality of the protein in the ration was decreased without change in its quantity; in period 3 the quality was increased above its original level

3. When a milking cow has been for a time on a ration inadequate in protein, the quantity of protein may be increased 90 to 100 per cent without an increase in plasma amino N. If the quality of the dietary protein is improved simultaneously with the increase in its quantity, the plasma amino N drops decidedly.

4. Under appropriate conditions the quality of the dietary protein affects decidedly the level of plasma amino N and the ration containing the more efficient protein mixture is associated with the lower level of plasma amino N.

5. The nutritional condition of an animal, as brought about by its dietary history, has a very great effect upon the level of plasma amino N associated with a given ration.

6. In the case of milking cows, where absorption is a fairly constant and con-

tinuous process and where there is a constant demand for energy for milk secretion, the level of plasma amino N is frequently more influenced by (a) the energy content of the ration, (b) the quality of the dietary protein, and (c) the nutritional condition of the animal than by the actual quantity of protein in the ration.

7. Under appropriate conditions very great changes may occur in the concentration of the free tryptophane of the blood, while at the same time little or no change occurs in the level of total amino N. These alterations in the composition of blood mixture of amino acids may continue for weeks. They occur when either the quantity or quality of the dietary protein is changed.

8. Sharp reductions in the quantity of energy in the ration or in either the quantity or quality of the protein are immediately followed by reductions in milk yield. The composition of the milk also undergoes changes which may reasonably be attributed to the changes in diet. When the original ration is again substituted for the reduced ration, there is a general tendency for the yield and composition of the milk to return to their original status. A study of these changes in the rations and milk, along with those occurring simultaneously in the amino N, tryptophane, and other blood constituents which have been determined in these experiments, justifies the working hypothesis that changes in dietary protein and energy affect milk secretion largely by inducing changes in the quantity and quality of the amino-acid mixture circulating in the blood plasma.

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